SCIENCE LIBRARY

AUG 19 1935

VOLUME 25 NUMBER 8

AUGUST, 1935

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

CONTENTS

Fusarium strains in relation to wilt of China aster. REGINA S. RIKER and L. R. JONES 733

Rhizoctonosis of potatoes grown under irrigation ..... L. A. SCHAAL 748

The nature of inhibition between certain fungi parasitic on citrus. JAIME GUISCAFRÉ ARRILLAGA 763

Acquired immunity from cucumber mosaic in zinnia ..... W. C. PRICE 776

Morphologic studies of Septoria lycopersici ..... HUBERT A. HARRIS 790

A phytophthora disease of peach seedlings ..... JOHN C. DUNEGAN 800

Summary of investigations with Ustilago striaeformis parasitizing some common grasses ..... W. H. DAVIS 810

Price { $6.00 per year, United States and dependencies,
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

COR. LIME AND GREEN STREETS, LANCASTER, PA.

Entered at the post-office in Lancaster, Pa., as second-class matter.

DIRECTORY OF ADVERTISERS

AMERICAN CYANAMID CO., 535 Fifth Ave., New York, N. Y.	AERO CYANAMID FOR SOIL STERILIZATION AND WEED CONTROL.
BAYER–SEMESAN CO., INC., DuPont Building, Wilmington, Del.	SEED AND SOIL TREATMENTS. NEW IMPROVED CERESAN. NEW IMPROVED SEMESAN JR.
BAUSCH & LOMB OPTICAL CO., 665 St. Paul St., Rochester, N. Y.	MICROSCOPES. MICROTOMES.
THE BOTANICAL REVIEW, N. Y. Botanical Garden, Bronx Park, N. Y.	A NEW REVIEW MAGAZINE.
CORNING GLASS WORKS, Corning, New York.	PYREX CYLINDRICAL JARS. PYREX PETRI DISHES.
CLAY–ADAMS CO., 25 East 26th St., New York, N. Y.	SLIDES AND COVER GLASSES. MICROSCOPIC PROJECTION AND DRAWING APPARATUS.
DIFCO LABORATORIES, 920 Henry St., Detroit, Mich.	DEHYDRATED CULTURE MEDIA.
THE GRASSELLI CHEMICAL CO., Cleveland, Ohio.	SPRAY MATERIALS AND DUSTS.
LINDER & COMPANY, INC., 298 North Beacon St., Brighton Station, Boston, Mass.	VAPORIZING SULFUR. SULFUR-NEBULATOR.
MERCK & CO., INC., Rahway, New Jersey.	COPPER OXIDE RED POWDER, CALOMEL, R. T. 50.
N. V. POTASH EXPORT M. Y., INC., 19 West 44th St., New York, N. Y.	AGRICULTURAL AND SCIENTIFIC BUREAU. POTASH FERTILIZER.
NIAGARA SPRAYER AND CHEMICAL CO., INC., Middleport, New York.	DUSTERS, DUSTS, AND SPRAYS. KOLOFOG, POMO-GREEN.
ROHM & HAAS & CO., INC., 222 W. Washington Square, Philadelphia, Pa.	RED COPPER OXIDE. FORM-O-FUME FORMALDEHYDE DUST.
THE ROESSLER & HASSLACHER CHEMICALS DEPT., E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.	P. A. C. FORMALDEHYDE.
SCIENCE PRESS, Lime and Green Sts., Lancaster, Pa.	PRINTERS OF SCIENTIFIC JOURNALS, BOOKS AND MONOGRAPHS.
TRIARCH BOTANICAL PRODUCTS, Geo. H. Conant, Ripon, Wis.	PRESERVED PLANTS. MICROSCOPE SLIDES.
PHYTOPATHOLOGICAL CLASSICS, H. H. Whetzel Mgr., Cornell University, Ithaca, N. Y.	NUMBERS 1, 2, 3, AND 4, PHYTOPATHOLOGICAL CLASSICS.
VIRGINIA SMELTING COMPANY, West Norfolk, Virginia.	SEED TREATMENT "VASCO 4."

PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

EDITORS

H. B. HUMPHREY, Editor in Chief, U. S. Department of Agriculture, Washington, D. C.
H. M. QUANJER, Editor for Europe, Instituut voor Phytopathologie, Wageningen, The Netherlands.
ANNIE RATHBUN GRAVATT, U. S. Department of Agriculture, Washington, D. C.
EUBANKS CARSNER, Citrus Experiment Station, Riverside, Calif.

ASSOCIATE EDITORS

JESSIE I. WOOD, U. S. Department of Agriculture, Washington, D. C.
B. F. LUTMAN, University of Vermont, Burlington, Vt.
LEE M. HUTCHINS, Fort Valley, Ga.
C. D. LA RUE, University of Michigan, Ann Arbor, Mich.
GLENN G. HAHN, Yale University, New Haven, Conn.
R. P. WHITE, State College of Agriculture and Mechanic Arts, New Brunswick, N. J.
F. O. HOLMES, The Rockefeller Institute for Medical Research, Princeton, N. J.
J. J. CHRISTENSEN, University Farm, St. Paul, Minn.
A. G. NEWHALL, Cornell University, Ithaca, N. Y.
ARTHUR H. EDDINS, Hastings, Fla.
G. B. SANFORD, Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton, Alberta, Canada.
F. L. DRAYTON, Central Experimental Farm, Ottawa, Ont., Canada.

BUSINESS MANAGEMENT

H. A. EDSON, Business Manager, U. S. Department of Agriculture, Washington, D. C.
R. S. KIRBY, Advertising Manager, Pennsylvania State College, State College, Pa.

NOTICES

Subscription price. $6.00 per year United States and dependencies; Canada $6.25; other countries $6.50; current single numbers 60 cents. The journal is issued monthly beginning with Volume VIII, January, 1918.

Advertisements. Rates may be secured from business management. No indorsement of any statement of claims made in advertisements is assumed by this journal or by The American Phytopathological Society.

Requests to supply copies of the journal must be made within 60 days from date of issue. Date of issue January 15 and monthly thereafter.

Separates. No gratis copies are supplied. A printed schedule of prices is submitted with the proof, and authors may secure separates by placing an order on the blanks provided when proof is returned.

Back Volumes IX to XXIV inclusive, (except Vol. IX, No. 1 and Vol. X, Nos. 1 and 4, which are not available) unbound at $8.00 per volume. All earlier volumes obtainable only in broken numbers.

Separate Numbers from Volumes IX to XXIV, $1.00 per copy; from Volumes I to VIII, $2.00 per copy. The following numbers of Volumes I to VIII, inclusive, are obtainable: Volume I, Nos. 1, 4, 5, 6 and index; Vol. II, Nos. 1, 5 and index; Vol. III, Nos. 4, 5, 6; Vol. VI, index only; Vol. VIII, Nos. 2 and 12 and index.

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity, and conciseness are essential. In form and style, such as punctuation, spelling and use of italics manuscripts should conform to the best usage in recent numbers of this journal. Manuscripts should be typed on one side of the paper, double spaced, and sent unfolded. All papers longer than four printed pages should include a summary. The name and address of the institution at which the work was done should be included at the end of the summary. All footnotes should be numbered consecutively with arabic numerals except in tables, where small letters should be used. Each table should have a heading stating clearly and concisely its contents. References to literature should be made as footnotes only when five or fewer citations are given. The simplified method of citing literature used in recent numbers of this journal should be followed. References in "Literature Cited" should be listed alphabetically and numbered consecutively.

Illustrations necessarily must be limited in number, and photographs, to reproduce satisfactorily, must be of the best quality. Authors desiring unusual numbers of illustrations or special types of illustrations will be asked to bear part of the expense. All illustrations are reproduced on the same grade of paper and will be run as text figures wherever possible. Plates should be used only for a number of small illustrations which can not be run conveniently as individual text figures. Individual illustrations within a plate should be designated by letters to avoid confusion with text figures which are designated by arabic numerals. Authors should arrange plates to conform to page size and shape (4½ inches by 7 inches) after making due allowance for reductions. Specific reference to each illustration must be made in the text. There should be a complete legend for each illustration. All legends should be on separate paper and not on the illustration itself.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

OFFICERS

President

H. T. GÜSSOW, Central Experimental Farm, Ottawa, Ont., Canada.

Vice-President

F. C. MEIER, U. S. Department of Agriculture, Washington, D. C

Secretary

H. P. BARSS, U. S. Department of Agriculture, Washington, D. C.

Treasurer

H. A. EDSON, U. S. Department of Agriculture, Washington, D. C.

Councilors

J. C. ARTHUR, Purdue University, La Fayette, Ind.
F. D. HEALD, State College of Washington, Pullman, Wash.
H. B. HUMPHREY, U. S. Department of Agriculture, Washington, D. C.
J. C. WALKER, University of Wisconsin, Madison, Wis.

Councilors Representing Divisions

T. E. RAWLINS, University of California, Berkeley, Calif. (Western Division).
V. H. YOUNG, University of Arkansas, Fayetteville, Ark. (Southern Division).

PHYTOPATHOLOGY

VOLUME 25 NUMBER 8

AUGUST, 1935

FUSARIUM STRAINS IN RELATION TO WILT OF CHINA ASTER[1]

REGINA S. RIKER AND L. R. JONES

(Accepted for publication December 27, 1934)

INTRODUCTION

The present publication, an abstract of which has already appeared (25), describes studies at the University of Wisconsin on the pathogenicity to China aster, *Callistephus chinensis* Nees, of various Fusarium strains from this country and abroad. These include both Fusaria that produce wilts of other hosts and Fusaria isolated from wilting asters. In addition, the behavior of the pathogenic forms has been tested toward host strains of known character, *i.e.*, not only toward those susceptible but also toward those resistant to the Beach and the Jackson isolations of *Fusarium conglutinans* Wr. v. *callistephi* Beach.

This paper is in continuation of one by Jones and Riker (13) dealing with the nature and control of the wilt and yellows of the China aster. That publication confirms the earlier work of Beach (4) and others that the wilt, caused by the vascular Fusarium, *F. conglutinans* v. *callistephi*, is wide spread and destructive in the United States, and indicates that, like other vascular fusarium diseases, this aster wilt yields to control through the selection of wilt-resistant host strains. As therein detailed, selections for wilt resistance against the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* were stabilized to the point of commercial acceptance in several basic colors and a fairly wide range of flower type and growth habit. Particular attention was paid to the American Branching type, most in use by the florists and home gardeners of the Wisconsin-Chicago district, in the important florists' colors, *viz.*, white, pink, rose, lavender, purple, and red, and to the Heart of France. Enough additional work was done with several other types of China aster (Royal, Comet, and American Beauty) to justify the conclusion that, in general, by repeated selection a commercially satisfactory degree of resistance may be found to this fusarium wilt. It also was pointed out in the same publication that these selected strains proved resistant not only in Wisconsin, but in such

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

widely scattered localities in this country as Washington, D. C., Yonkers, New York, and El Monte, California. Favorable reports on the behavior of the strains in Ithaca, New York (22, 23, and 24), and West Chicago, Illinois (2), have appeared in the literature.

PATHOGENICITY OF VARIOUS FUSARIUM STRAINS TO CHINA ASTER

Pathogenicity of Various Vascular Fusaria that Induce Wilts of Other Hosts

The serious wilt-inducing Fusaria are, in general, placed by Wollenweber[2] in one section, *Elegans*. Because of the mutual resemblances of these pathogens, as well as of the associated diseases, it seemed worth while to test the pathogenicity to China aster of as many of these from other hosts as were available. Three series of trials were made, all in greenhouse culture. For these, seedlings of an aster strain susceptible to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* were grown in flats of wilt-free soil. When the second leaf appeared, they were transplanted to 5-inch pots, 6 to each pot. At the time of transplanting the soil was inoculated by placing a small section of an agar plate culture of the organism to be tested in the hole into which the plant was dropped. In every series 2 pots were used for each organism. Thus, in all, 36 plants were tested with each Fusarium. The Fusaria of the section *Elegans* used,[3] with their classification and host source, were:

Sub-section *Orthocera*
- *F. conglutinans* Wr. from cabbage
- Do. ..... v. *betae* Stew. from sugar beet
- *F. orthoceras* App. et Wr. v. *pisi* Wade from pea

Sub-section *Constrictum*
- *F. bulbigenum* Cke. et Mass. v. *niveum* (Erw. Sm.) Wr. from watermelon
- Do. ..... v. *tracheiphilum* (Erw. Sm.) Wr. from cowpea

Sub-section *Oxysporum*
- *F. oxysporum* Schl. from cabbage
- Do. ..... beet
- Do. ..... potato
- *F. vasinfectum* Atk. from cotton

In addition, an unidentified Fusarium from celery was used.

In these trials, none of the organisms tested induced wilt of China aster. With each of the organisms listed above, the plants remained 100 per cent

[2] The nomenclature and classification of the Fusaria used throughout this paper are those of Wollenweber (40).

[3] The cultures employed were furnished by various members of the Department of Plant Pathology at the University of Wisconsin with the following exceptions. The 3 strains of *Fusarium oxysporum* were supplied by H. W. Wollenweber. *F. vasinfectum* was sent by V. H. Young. The assistance of these colleagues is gratefully acknowledged.

healthy at the end of 6 weeks, while in the controls, inoculated with the Beach isolation of *F. conglutinans* v. *callistephi*, only 8 per cent of the plants were healthy at the end of the same period.

These negative results on China aster confirm previous reports in the cases of *F. conglutinans* (11, 12, and 31) and *F. conglutinans* v. *betae* (31).

Pathogenicity of Various Fusaria from China Aster

A survey of the literature, supplemented by observations of L. R. Jones and by correspondence, indicates the almost universal occurrence of fusarium aster wilt wherever the culture of the host plant has been long established. Its widespread distribution in the United States has been indicated by the writers (13). In Canada the disease has been particularly studied in Ontario (11) and New Brunswick (15). In Europe it has been reported from Austria (7); Czecho-Slovakia (30) and (3); Denmark (9); England (18) and (19); Germany (many references including those of Gante (8) and Wollenweber (41)); Greece (1); Holland (26) and (32); Italy (21), (33), and (34); and Switzerland (20) and (16). In the summer of 1930 L. R. Jones observed what appeared to be fusarium wilt of aster under the guidance of H. W. Wollenweber in Germany, and Elias Melin in Sweden. The disease has been reported also from South Africa (35), (36), and (37); New Zealand (14) and (5); and Tasmania (17). In Australia a wilt of asters has been noted (6); and D. B. Adam, in correspondence, has referred to a wilt in China asters caused, he believed, by *Fusarium conglutinans* v. *callistephi*. In 1931 L. R. Jones was shown evidence of fusarium wilt in Japan by T. Fukushi and T. Hemmi.

The identity of the Fusarium involved has not always been determined. Several species besides *Fusarium conglutinans* v. *callistephi* have been listed as associated with diseased asters. However, the pathogenicity of many of these species has not been established. Wollenweber (39) listed, as found on China aster, *F. flavum* (Fr.) Wr., *F. dimerum* Penz., *F. orthoceras* Ap. et Wr., *F. conglutinans* Wr., *F. polymorphum* Matr., *F. culmorum* (W. Sm.) Sacc., *F. graminum* Cda., and *F. pyrochroum* (Desm.) Sacc. He pointed out that it had not yet been determined which of these were harmful. Gante (8) reported the isolation of *F. graminum*, *F. polymorphum*, *F. culmorum*, *F. falcatum* Ap. et Wr., and *F. dimerum* from diseased asters. He added that inoculation experiments were tried with *F. graminum*, *F. culmorum*, and *F. falcatum* and negative results obtained. Besides *F. conglutinans* v. *callistephi*, Jackson (11) found four other Fusaria, isolated from diseased asters in Canada, pathogenic to the host. Only one of these was identified, namely, *F. angustum* Sherb. *F. culmorum* isolated from asters was found nonpathogenic to asters. White (38), in considering the wilt of the carnation, reported that strains of *F. culmorum*

pathogenic to carnations caused aster wilt. Baudys (3) stated that the fusarium disease of asters in Czecho-Slovakia was ordinarily caused by *F. pyrochroum* and *F. conglutinans* v. *callistephi*. However, H. W. Wollenweber identified *F. solani* (Mart.) v. *Martii* (App. et Wr.) f. *1* Wr. and *F. scirpi* Lamb. et Fautr. v. *acuminatum* (Ell. et Ev.) Wr. on specimens sent to him. Wager (36) established *F. conglutinans* v. *majus* Wr. as the cause of aster wilt in South Africa. Wollenweber (41) mentioned *F. conglutinans* v. *majus* and *F. oxysporum* Schl. f. *6* Wr. as now and then isolated from wilted asters in Germany. He also listed *F. culmorum*, *F. sambucinum* Fckl., *F. lateritium* Nees, and *F. herbarum* (Cda.) Fr., with varieties, as observed on aster with harmful effect yet to be determined.

In the present studies the pathogenicity to China aster of various strains of Fusaria isolated from diseased asters was tested in the greenhouse and to a lesser extent in out-door cloth-covered houses. The strains of Fusarium used came from Europe and Japan and were compared in behavior with two American isolations of *F. conglutinans* v. *callistephi*, namely, the Beach isolation from Michigan and the Jackson isolation from Ontario.[4]

To test the pathogenicity of these Fusaria in the greenhouse, the method described in the preceding section was modified slightly. The seedlings were transplanted to flats instead of pots, each flat containing 48 plants. In the cases where negative results were obtained the trials were repeated twice, thus testing 144 plants with each organism. When positive results were obtained, further studies were conducted as described in the following section. Out-door trials in cloth-covered enclosures of certain of the Fusaria were made during the summer of 1931, using 50 plants with each organism.

The results of both greenhouse and out-door, cloth-covered-cage tests are summarized in table 1. In every instance the cloth-covered-cage results agreed with those obtained in the greenhouse. This table shows that, in the case of both the European and the Japanese Fusaria, some forms isolated from diseased asters proved pathogenic, while some did not. Of the pathogenic forms all but one, namely, *F. lateritium* Nees v. *fructigenum* (Fr.) Wr., belonged in the section *Elegans*. However, not all of the Fusaria of this section, isolated from diseased asters, proved pathogenic to aster.

Comparison with the results of the preceding section indicates that Fusaria, closely related morphologically and culturally, differ markedly in

[4] The writers wish to express their appreciation of the kind cooperation of several colleagues. The Beach and the Jackson isolations were sent by these men, respectively, and are the same as those used in the investigations reported in the earlier publication (13). The European forms were furnished and identified by H. W. Wollenweber. The Japanese forms were sent by T. Fukushi (J. A.) and T. Hemmi (J I, J II, J III, J VI), and the pathogenic strains identified by H. W. Wollenweber.

TABLE 1.—*Trials of pathogenicity to China aster of strains of Fusaria from wilting asters*

Fusaria		Pathogenicity[a]	
Species and culture number[b]	Source	Greenhouse	Outdoor cage
Elegans			
Orthocera			
F. conglutinans Wr. v. *callistephi* Beach (305)	Michigan	+	+
Do. (435)	Ontario	+	+
Do. (1472)	Germany	+	+
Do. v. *majus* Wr. (3206)	do	+	+
Do. (4422)	do	+	...
Do. (J. A)	Japan	+	+
Do. (J. VI)	do	+	+
Oxysporum			
F. oxysporum Schl. f. *6* Wr. (4179)	Germany	+	+
Do. (4200)	do	+	...
Do. (4497)	do	+	...
Do. (4506)	do	+	...
Do. (J. I)	Japan	+	...
Do. v. *aurantiacum* (Lk.) Wr. (4453)	Germany	0	...
F. redolens Wr. (4428)	do	0	...
Do. (4495)	do	0	...
F. vasinfectum Atk. v. *lutulatum* (Sherb.) Wr. (4494)	do	0	...
Roseum			
F. herbarum (Cda.) Fr. f. *1* Wr. (4220)	Sweden	0	...
Do. f. *2* Wr. (2826)	Germany	0	...
Do. v. *avenaceum* (Fr.) Wr. (4254)	Sweden	0	...
Gibbosum			
F. scirpi Lamb. et Fautr. v. *acuminatum* (Ell. et Ev.) Wr. (1725)	Germany	0	...
F. equiseti (Cda.) Sacc. (3250)	do	0	...
Do. (4229)	Sweden	0	0
Discolor			
F. culmorum (W. G. Sm.) Sacc. (4420)	Germany	0	...
Lateritium			
F. lateritium Nees v. *fructigenum* (Fr.) Wr. (4421)	do	+	+
Martiella			
F. solani (Mart.) v. *martii* (App. et Wr.) Wr. (4230)	Sweden	0	...
UNIDENTIFIED			
Fusarium sp. (J. II)	Japan	0	...
Do. (J. III)	do	0	...

[a] + indicates that the fungus strain was pathogenic; 0, nonpathogenic; ..., not tested.

[b] The culture numbers used in all tables are those of H. W. Wollenweber for the European forms, those of T. Hemmi for the Japanese forms designated by Roman numerals, and those of Wisconsin for 305, 435, and J. A.

pathogenicity. As evidence of this, *F. conglutinans* v. *callistephi* and *F. conglutinans* v. *majus* were pathogenic to China aster, while *F. conglutinans* and *F. conglutinans* v. *betae* were not. Likewise, *F. oxysporum* from cabbage, potato, and beet did not induce wilt of aster, while 5 strains of *F. oxysporum* f. *6*, isolated from diseased asters, proved pathogenic. These results, therefore, so far as they go, are in accord with the concept of narrowly limited host specialization in the parasitism of Fusaria of the section *Elegans*.

RESISTANCE TO VARIOUS FUSARIA OF STRAINS OF CHINA ASTER SUSCEPTIBLE AND WILT-RESISTANT WITH RESPECT TO F. CONGLUTINANS V. CALLISTEPHI

Representatives of the aster strains, which had been previously selected for wilt resistance to both the Beach and the Jackson isolations of *Fusarium conglutinans* v. *callistephi* (13), were tested for wilt resistance toward each of the other Fusaria found pathogenic to aster in the present studies. In the following discussion the terms susceptible and resistant refer to the behavior of the aster strains with respect to the above isolations of *F. conglutinans* v. *callistephi*. Trials with the entire series of pathogenic Fusaria were made in the greenhouse and with certain of the forms in out-door cloth-covered enclosures.

Greenhouse Trials

The following procedure was used for the greenhouse studies. Seedlings of an aster strain susceptible to *Fusarium conglutinans* v. *callistephi*, and of 3 strains resistant to this organism were grown in flats of wilt-free soil. When the second leaf appeared the plants were transplanted to flats, 8 rows of 6 plants each, 2 rows to an aster strain. Thus each flat contained 12 susceptible and 36 resistant plants. Inoculations were made at the time of planting as described in an earlier section. Three series of trials were run, a total of 36 susceptible and 108 resistant plants being tested with each organism.

The results of the greenhouse trials are summarized in table 2. To all of the Fusaria pathogenic to aster that were used in these trials, with the exception of the Japanese strain of *F. oxysporum* f. *6* (J. I), the susceptible strain of aster was relatively susceptible, while the 3 wilt-resistant strains proved relatively resistant. Figure 1 is representative of the results obtained. In other words, with one exception, these various Fusarium strains behaved similarly in respect to pathogenicity to the isolations of *F. conglutinans* v. *callistephi* used. Another exception will be mentioned later in this paper. It should be noted that the Fusaria thus agreeing closely in their pathogenicity and inducing essentially similar wilt diseases represent different varieties, species, and even sections as regards their morphological

TABLE 2.—*Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Greenhouse trials*

Fusaria Species and culture number	Susceptible asters	Resistant asters
	Per cent resistant	Per cent resistant
F. conglutinans v. *callistephi* (305)	14	94
Do. (435)	8	88
Do. (1472)	14	97
Do. v. *majus* (3206)	14	97
Do. (4422)	47	95
Do. (J. A)	50	92
Do. (J. VI)	25	83
F. oxysporum f. *6* (4179)	28	85
Do. (4200)	17	93
Do. (4497)	28	98
Do. (4506)	25	93
Do. (J. I)	58	58
F. lateritium v. *fructigenum* (4221)	25	93

and cultural characters. On the other hand, in the same "forma" (*F. oxysporum* f. *6*) different isolations behaved differently toward the wilt-resistant asters. Thus the morphological and cultural characteristics were not an index of pathogenicity.

Out-door Cage Trials

Out-door cloth-covered-enclosure tests with certain of the pathogenic Fusarium strains were made in the summers of 1931 and 1932. Seedlings of the aster strains to be tested were grown in flats of wilt-free soil. When the second leaf appeared they were transplanted to other flats of wilt-free soil. When the plants were 9 weeks old, they were planted in the field. Inoculations were made at this time. The inoculum had been prepared by transferring the organism to quart jars half-full of a sterile mixture of corn meal and sand. These were incubated at room temperature for 15 days. Then the inoculated corn meal and sand were mixed with wilt-free soil in the proportions of 1 to 5 and the whole incubated at room temperature for 7 days. At the time of planting approximately 100 grams of this inoculated soil was placed around each plant. In 1931 the asters were grown in ground where no asters had previously been planted, using 10 susceptible plants and 30 resistant plants representing 3 strains, with each organism. In 1932 the asters were grown in plots of steamed soil, 30 susceptible plants representing 2 strains, and 60 resistant plants representing 3 strains, being tested with each Fusarium. The necessity for growing the

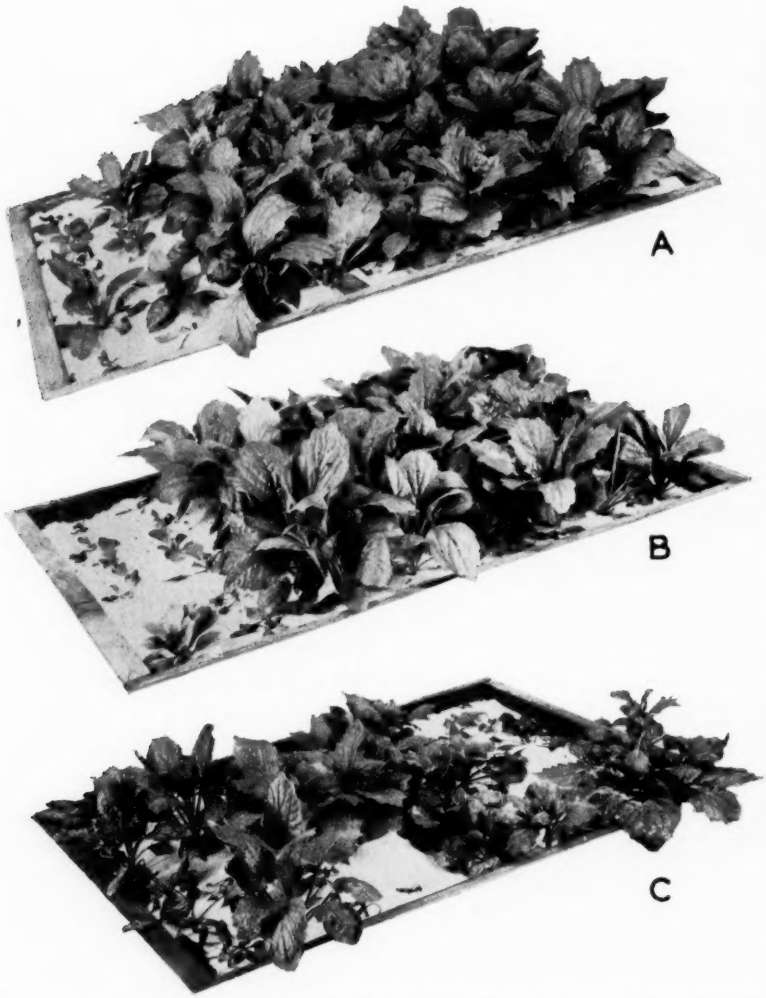


FIGURE 1. Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Greenhouse tests in shallow boxes or "flats."

The soil in each box was inoculated with a different Fusarium, and planted alike with 8 rows of asters, 6 plants to each row. The 2 rows at left in each box were susceptible to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* and the 6 rows at right were resistant, including 2 rows each of 3 different aster strains.

The organisms used in the soil inoculation were:

A. *F. conglutinans* v. *callistephi* (305). This is the Beach isolation of the aster-wilt organism. Note the general sickness of the susceptible asters, 2 rows at left, and contrasting vigor of the resistant asters at right. The behavior of this organism is also featured in plate I, C.

B. *F. conglutinans* v. *majus* (3206). This isolation from Germany has, in all Wisconsin tests, shown essentially like pathogenicity with the Beach isolation of *F. conglutinans* v. *callistephi* (Figure 1, A; Plate I, C). The behavior of this organism is also featured in plate I, C.

C. *F. oxysporum f. 6* (J. I). This strain from Japan is quite as pathogenic to the resistant asters, at right, as to the susceptible ones at left.

Before photographing, the soil was covered with white sand in order to secure clearer differentiation of the sickly plants.

asters under cloth (13) so limited the available space as to preclude using larger numbers in the trials and employing the entire series of pathogenic Fusaria in these studies.

The results are shown in table 3 and plate I. The behavior of the various Fusaria during the two seasons is essentially similar and agrees

TABLE 3.—*Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Out-door cage trials*

Fusaria Species and culture number	Susceptible asters	Resistant asters
	Per cent resistant	Per cent resistant
SEASON OF 1931		
F. conglutinans v. *callistephi* (305)	30	93
Do. v. *majus* (3206)	30	100
F. oxysporum f. *6* (4200)	10	87
F. lateritium v. *fructigenum* (4221)	20	93
SEASON OF 1932		
F. conglutinans v. *callistephi* (305)	0	92
Do. v. *majus* (3206)	2	93
Do. (J. A)	0	92
Do. (J. VI)	2	97
F. oxysporum f. *6* (4200)	0	93
F. lateritium v. *fructigenum* (4221)	8	97
Noninoculated	93	98
Do.	88	98

with that in the greenhouse trials. Since the Japanese strain of *Fusarium oxysporum* f. *6* was not used in the out-door cage tests, the susceptible aster strains were relatively susceptible to all the pathogens, while the resistant strains proved relatively resistant to all.

Results of Collaborators

During the past few years the wilt-resistant aster strains developed at Wisconsin have been tested abroad as well as in various localities in this country. Several references to the behavior of these strains outside the United States have appeared in the literature. MacLeod (15) in New Brunswick, Canada, using 77 strains of aster, obtained 7 to 100 per cent infection in soil infested with *Fusarium conglutinans* v. *callistephi*, except with 5 Wisconsin strains that proved completely resistant to wilt on the soil used. Ogilvie and Mulligan (19), in England, tested 5 Wisconsin varieties, other wilt-resistant American varieties, and the common English market varieties on soil infected with *F. conglutinans* v. *majus*. The English market varieties gave almost 100 per cent wilt. On the other hand, the

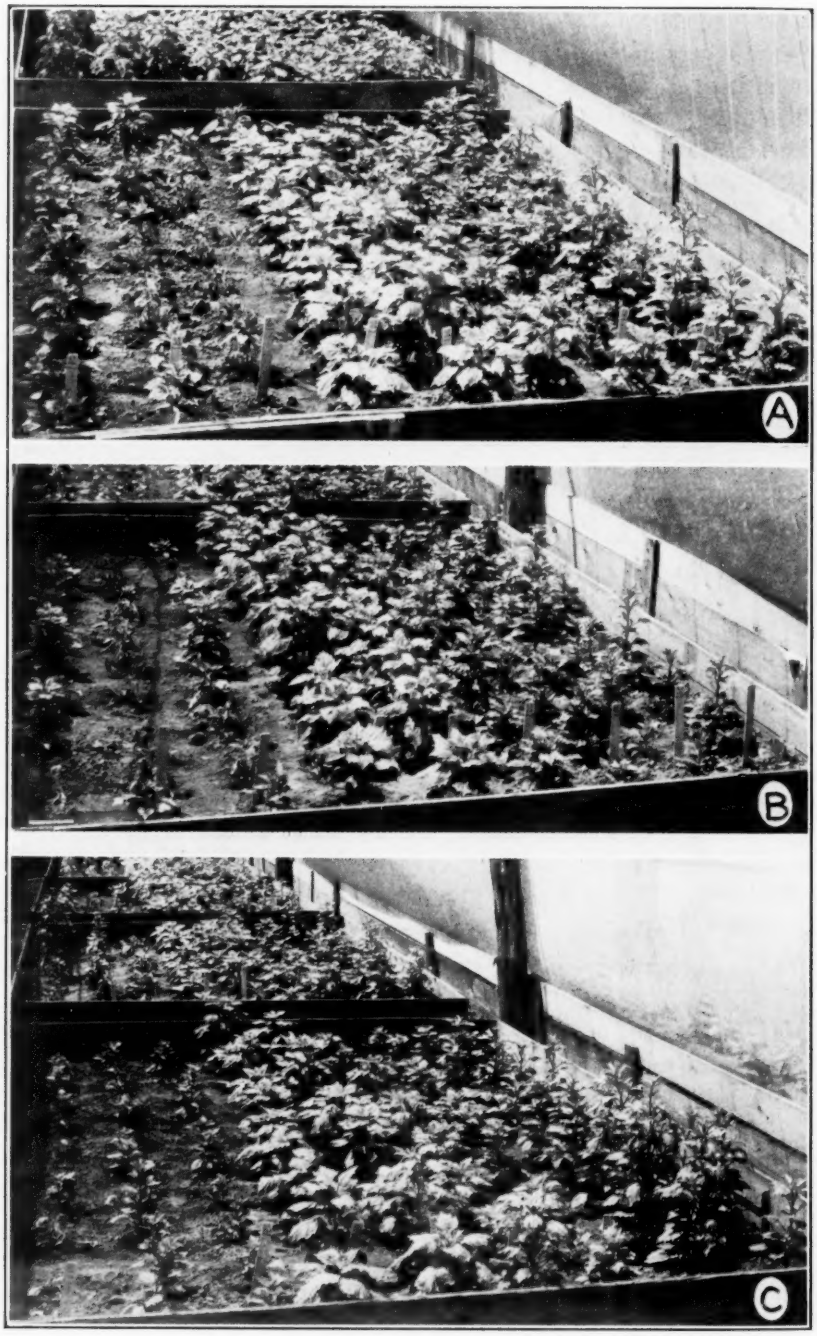


PLATE I

Wisconsin aster strains showed no wilt and the other American resistant varieties averaged 10 per cent wilt. On soil infected with *F. conglutinans* v. *callistephi*, where a planting of Giant Comet White had been severely attacked, 4 Wisconsin varieties showed almost no wilt. Wager (36), in South Africa, where the wilt is caused by *F. conglutinans* v. *majus*, found the 7 Wisconsin varieties moderately resistant, since an average of 47 per cent wilt was obtained, whereas the control variety showed 99 per cent.

These reports are in agreement with the results detailed in this paper that the Wisconsin wilt-resistant aster strains may prove more resistant than commercial susceptible strains[5] not only toward *F. conglutinans* v. *callistephi*, but also toward *F. conglutinans* v. *majus*.

Indiana Situation

While, in general, reports regarding the selected Wisconsin aster strains were favorable as to their resistance to fusarium wilt, one notable exception occurred in this country. E. R. Honeywell, in trials at the Purdue University (Indiana) Experiment Station, found supposedly resistant strains

[5] There are now on the market a number of commercial wilt-resistant strains that have been developed by selection from the Wisconsin or other aster strains largely by George J. Ball, West Chicago, Illinois, and Bodger Seeds, Ltd., El Monte, California.

PLATE I. Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Out-door cage trials 1932.

The steamed soil in each compartment, except the control, was inoculated with a different isolation of Fusarium, and planted alike with 9 rows of asters, 10 plants to each row. The 3 rows at left in each compartment were susceptible to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* and the 6 rows at right were resistant, including 2 rows each of 3 different aster strains.

The organisms used in the soil inoculation were:

A. *F. lateritium* v. *fructigenum* (4221) from Germany in front plot. A little of the noninoculated control compartment shows in the background.

B. *F. oxysporum* f. *6* (4200) from Germany in front plot. The pathogenicity is essentially like that of the Beach isolation of *F. conglutinans* v. *callistephi* shown in figure 1, A and plate I, C, and in marked contrast with the Japanese isolation of *F. oxysporum* f. *6* (J. I) shown in figure 1, C. In the back plot *F. lateritium* v. *fructigenum* (4221) was used (Plate I, A).

C. In plots from front to back:

F. conglutinans v. *callistephi* (305) from Michigan. This is the Beach isolation and its behavior is also featured in figure 1, A.

F. conglutinans v. *majus* (3206) from Germany. The behavior of this organism also is featured in figure 1, B.

F. oxysporum f. *6* (4200) from Germany (Plate I, B).

F. lateritium v. *fructigenum* (4221) from Germany (Plate I, A).

Note that in all cases the organisms showed essentially like pathogenicity. The susceptible asters to the left were generally sick, while the resistant asters to the right were almost all healthy.

extremely susceptible to wilt. Brief discussions of his results have appeared (27, 28, 29, and 10). Riker saw Honeywell's trials in Indiana in the summer of 1932. Subsequent greenhouse tests at Wisconsin confirmed the Indiana results. In these, two methods were employed (1) using aster-sick Indiana soil; (2) using soil inoculated with organisms isolated from the Indiana soil.[6] In both cases the results were the same. The Indiana fungus was fully as pathogenic with the resistant Wisconsin aster strains as with the susceptible aster strains. This exceptional Indiana pathogen was sent to H. W. Wollenweber, who identified it as *Fusarium conglutinans* v. *majus*. It is to be noted that isolations of *F. conglutinans* v. *majus* from Germany and Japan in trials reported in this paper, and from England (19), and South Africa (36) in other experiments, have in general agreed in pathogenic behavior with the typical aster-wilt organism, *F. conglutinans* v. *callistephi*. As mentioned earlier, one of the Japanese isolations likewise proved pathogenic to the Wisconsin wilt-resistant aster strains. It was, however, rather less virulent than the Indiana fungus. This Japanese fungus has been identified by H. W. Wollenweber as *F. oxysporum* f. *6*. The outcome of a trial with this organism is illustrated in figure 1, C. It is noteworthy that here, again, is evidence that like types of pathogenicity may occur with Fusaria differing in morphological or cultural characters.

While these results are limited in extent, they are fully convincing that aster wilt Fusaria occur to which the Wisconsin selected aster strains are highly susceptible. Experience with the vascular Fusaria, in general, would encourage the expectation that strains of aster may be found adequately resistant to this type of pathogen. Wisconsin conditions have not permitted continued search along this line. This can best be undertaken wherever the pathogen is detected locally, as in Indiana or Japan. The reports from Indiana, cited above, show that progress already is being made by E. R. Honeywell in this direction.

DISCUSSION

The experiments outlined in this paper indicate that morphological and cultural characters and pathogenicity among the Fusaria are not necessarily interrelated. Evidence for this was obtained in several ways. Fusaria closely related according to classification differed in their ability to attack the China aster. For example, *Fusarium conglutinans* v. *callistephi* and *F. conglutinans* v. *majus* were pathogenic to aster while *F. conglutinans* and *F. conglutinans* v. *betae* were not. Likewise, different isola-

[6] The writers wish to express appreciation of the kind assistance both of E. R. Honeywell who furnished samples of aster-sick soil from which isolations were made at Wisconsin, and of R. W. Samson, who supplied cultures of organisms isolated from diseased asters at Lafayette, Indiana.

tions of the same fungus differed in relative pathogenicity. An instance of this was *F. conglutinans* v. *majus*. The Wisconsin resistant asters were relatively resistant to isolations of this fungus from Germany and Japan, but extremely susceptible to an isolation from Indiana. On the other hand, Fusaria classified as of different varieties, species, and even sections behaved similarly as to pathogenicity. Most isolations of *F. conglutinans* v. *majus*, *F. oxysporum* f. *6*, and *F. lateritium* v. *fructigenum* used, agreed with the Beach isolation of *F. conglutinans* v. *callistephi* in that the Wisconsin resistant asters proved relatively resistant and the susceptible asters relatively susceptible.

SUMMARY

1. Pathogenicity tests with 10 Fusaria of the section *Elegans* that induce wilts on other hosts gave negative results with China aster.

2. Pathogenicity tests with 27 strains of Fusaria isolated from wilting asters showed that: (a) Some of the Fusaria were pathogenic to aster while some were not. (b) Of the pathogenic forms, most belonged to the section *Elegans*, but *Fusarium lateritium* v. *fructigenum* did not. (c) Not all of the Fusaria of the section *Elegans* isolated from diseased asters were pathogenic to aster.

3. Fusaria closely related morphologically and culturally, *i.e.*, merely varieties of the same species, differed in their ability to induce wilt of aster.

4. Several Fusaria (*F. conglutinans* v. *majus*, *F. oxysporum* f. *6*, and *F. lateritium* v. *fructigenum*), pathogenic to aster, but, according to standard methods, classified as a different variety or species or even in a different section, induced a wilt of aster essentially similar to that caused by the type strain of *F. conglutinans* v. *callistephi*. This was true for most of the isolations of these Fusaria tested not only as to general symptoms, but also as to relative pathogenicity. Asters both susceptible and wilt-resistant to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi*, proved, respectively, susceptible and wilt-resistant to a number of these other Fusaria.

5. Two exceptions were found to this agreement as to relative pathogenicity. One of these from Indiana was identified by H. W. Wollenweber as *F. conglutinans* v. *majus*, the other from Japan as *F. oxysporum* f. *6*. These 2 isolations caused as much wilt among the aster strains resistant to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* as among those susceptible. Isolations of each of these Fusaria from other sources behaved essentially like the Beach isolation of *F. conglutinans* v. *callistephi*.

6. Thus the evidence seems to justify the conclusion that (a) the morphological and cultural characters of the Fusaria associated with aster wilt

are not a reliable index of pathogenicity, and (b) that essentially the same type of aster wilt may result from the attack of Fusaria classified as of different varieties, or species, or even sections.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

LITERATURE CITED

1. ANAGNOSTOPOULOS, PANOS TH. Some diseases of fruit trees, vegetables and flowers, due to species of fungi of the genus Fusarium, met with in Greece. 23 pp. A. B. Pascha, Athens. 1933.
2. BALL, GEORGE J. Asters under tent safe from hopper. Florists' Rev. **66** (1718): 23–26. 1930.
3. BAUDYS, E. Škodliví činitelé rostlin okrasných. Ochrana Rostlin **12**: 58–63. 1932.
4. BEACH, WALTER S. The Fusarium wilt of China aster. Mich. Acad. Sci. Rpt. **20**: 281–308. 1918.
5. CUNNINGHAM, G. H. Additions to the recorded plant-diseases of New Zealand. New Zeal. Jour Agr. **42**: 305–306. 1931.
6. DARNELL-SMITH, G. P. Biological branch. New So. Wales Dept. Agr. Ann. Rpt. 1920–1921: 27. 1922.
7. FISCHER, R. Fuszkrankheit der Sommerastern. Gartenwelt **34**: 636–637. 1930.
8. GANTE, TH. Untersuchungen über Welkekrankheiten der Sommeraster. Ztschr. Pflanzenkr. **36**: 72–79. 1926.
9. GRAM, ERNST, and MATHIAS THOMSEN. Oversigt over Sygdomme hos Landbrugets og Havebrugets Kulturplanter i 1925. Tidsskr. Planteavl. **33**: 84–152. 1927.
10. HONEYWELL, E. R. Aster culture. Purdue Univ., Agr. Expt. Sta. Circ. 200. 1933.
11. JACKSON, A. B. The Fusarium wilt of China asters. Sci. Agr. **7**: 233–247. 1927.
12. JONES, L. R., and REGINA S. RIKER. Studies upon the Fusarium wilt of China aster. (Abst.) Phytopath. **18**: 150. 1928.
13. ————, and ————. Wisconsin studies on aster diseases and their control. Wis. Agr. Expt. Sta. Res. Bul. 111. 1931.
14. KIRK, T. W. Division of Biology and Horticulture. New Zeal. Dept. Agr. Ann. Rpt. **16**: 97–162. 1908.
15. MACLEOD, D. J. Aster wilt and aster yellows investigations in disease resistance. Canada Dept. Agr., Div. Bot., Rpt. Dom. Bot. 1930: 22–23. 1931.
16. MÜLLER-THURGAU, H., A. OSTERWALDER, and G. JEGEN. Pflanzenphysiologische und pflanzenpathologische Abteilung. Landw. Jahrb. Schweiz. **36**: 774–784. 1922.
17. NICHOLLS, H. M. Annual report of the government microbiologist. Tasmania Agr. and Stock Dept. Rpt. 1924–1925: 4. 1925.
18. OGILVIE, LAWRENCE. Observations on the diseases of market-garden crops in the Vale of Evesham. Agr. and Hort. Res. Sta., Long Ashton, Bristol, Ann. Rpt. 1929: 149–154. [1930].
19. ————, and B. O. MULLIGAN. The Fusarium wilt of China asters in England. Gard. Chron. **95**: 215–216. 1934.
20. OSTERWALDER, A. Die Fusarium-Krankheit bei Astern. Landw. Jahrb. Schweiz **24** (1910): 246–247. 1911.
21. PETRI, L. Rassegna dei casi fitopatologici osservati nel 1930. Bol. R. Staz. Patol. Veg. **11**: 1–50. 1931.
22. POST, KENNETH. The use of cheesecloth in aster culture. Cornell Ext. Bul. 212: 33–40. 1931.

23. POST, KENNETH. House of cloth for outdoor crops. Florists' Rev. **69** (1786): 17–19. 1932.
24. ————. Cut-flower production under cloth. Cornell Ext. Bul. 252. 1933.
25. RIKER, REGINA S., and L. R. JONES. Fusarium strains in relation to wilt of China aster. (Abst.) Phytopath. **23**: 29–30. 1933.
26. S[CHENK], P. J. De zwam Fusarium. Floralia **47**: 615. 1926.
27. SKINNER, J. H., and H. J. REED. Report of the director. Purdue Univ., Agr. Expt. Sta. Ann. Rpt. **44**. 87 pp. 1931.
28. ————, and ————. Report of the director. Purdue Univ., Agr. Expt. Sta. Ann. Rpt. **45**. 91 pp. 1932.
29. ————, and ————. Report of the director. Purdue Univ., Agr. Expt. Sta. Ann. Rpt. **46**. 82 pp. 1933.
30. SMOLÁK, JAR. Zprava o činnosti stanice pro choroby rostlin při st. vyašše škole ovocnicko-vinařkské a zahradnicke na Mělnice za rok 1923 a 1924. Ochrana Rostlin **5**: 41–46. 1925.
31. STEWART, DEWEY. Sugar-beet yellows caused by Fusarium conglutinans var. betae. Phytopath. **21**: 59–70. 1931.
32. VAN DER VEEN, RUTGER. Onderzoekingen over Tracheomycosen. Proefschr., Univ. Utrecht. 89 pp. Hollandia Drukkerij, Baarn. 1930.
33. VOGLINO, P. Le macchie brune degli astri Cina. La Difesa delle Piante **8** (6): 1–3. 1931.
34. VOGLINO, PIERO. Ricerche sulla Fusariosi degli Astri della Cina. Reprinted from Ann. R. Accad. Agr. Torino **75** (1932): 22 pp. 1933.
35. WAGER, VINCENT A. Wilt disease of asters and tests with resistant varieties. Pretoria Gard. **15** (166): 5–7. 1932a.
36. ————. Aster wilt in South Africa. So. African Jour. Sci. **29**: 301–312. 1932b.
37. ————. The wilt disease of asters. Farming in So. Africa **8**: 122–123. 1933.
38. WHITE, H. L. Wilt disease of the carnation. Expt. and Res. Sta., Cheshunt, Herts., Ann. Rpt. **14** (1928): 62–75. 1929.
39. WOLLENWEBER, H. W. Die Gattung Fusarium. In Sorauer, *et al.* Handbuch der Pflanzenkrankheiten. Aufl. 4, Bd. 3, pp. 164–185. Berlin. 1923.
40. ————. Fusarium-Monographie. Fungi parasitici et saprophytici. Ztschr. Parasitenk. **3**: 269–516. 1931.
41. ————. Die Gattung Fusarium. In Sorauer, *et al.* Handbuch der Pflanzenkrankheiten. Aufl. 5, Bd. 3, pp. 732–819. Berlin. 1932.

RHIZOCTONOSIS OF POTATOES GROWN UNDER IRRIGATION

L. A. Schaal

(Accepted for publication Dec. 8, 1934)

INTRODUCTION

The rhizoctonia disease of potatoes, caused by *Corticium vagum* Berk. and Curt., is still of major importance in the potato-growing areas of Colorado. Both stem and tuber infection are important phases of the disease. Stem infection includes the underground area of the plant. Under favorable conditions infection may involve the whole root system. Under normal growing conditions it is not serious in the expression of symptoms, but the loss due to reduced vitality of the plant and subsequent decrease in yield often is underestimated.

In the warmer soils of the irrigated areas, stem infection is seldom a limiting factor in potato culture. This type of infection often causes serious losses in the early crop and in those grown at high altitudes in the mountain valleys, where soil temperatures remain relatively low throughout the growing season.

In the irrigated areas of Colorado a wide variation of physical factors exists. The large area located east of the mountain range extending from north to south through the state is commonly called the eastern slope or the plains area. Certain sections close to this mountain range exist as cultivated farms only through the aid of irrigation water. Summer temperatures are usually high during the day but comparatively low at night. Two crops of potatoes, with reference to time of planting, are grown. The early crop consists of the varieties Bliss Triumph, Irish Cobbler, and Early Ohio, which make up the bulk of the acreage, and is planted between April 1 and May 10. This early crop matures marketable potatoes from July 15 to August 15. The late or main crop consists chiefly of two varieties, Rural New Yorker and Bliss Triumph, and is planted between May 25 and June 15. This crop is harvested from September 15 to October 30.

Varieties and planting dates in the mountain valleys vary with the location of the area in which the potatoes are grown. Many of the higher valleys are not free from frost until June 1. The soil usually remains cool and wet throughout the growing season and it is here that Rhizoctonia does most damage to the underground portions of the potato stems.

Observations and experiments have shown that stems of the early-crop potatoes often become infected with Rhizoctonia. In many cases the young sprouts are killed by the fungus before they reach the surface of the soil. Very little stem infection is found on the late- or main-crop potatoes of the

Greeley, Colorado, area. The soil temperature is quite high by June 1, and rapid germination follows planting of the seed. Tuber infection is rarely a factor on the early crop. Tubers harvested in September often show heavy infection.

With these facts in mind the writer undertook a series of experiments designed to determine the relation of temperature and moisture to infection of potatoes by Rhizoctonia. The experiments were undertaken with the hope of obtaining information that would be of value in formulating certain control measures based on cultural practice along with certain seed treatments.

REVIEW OF LITERATURE

A survey of the literature reveals the fact that rhizoctonosis is one of the most common diseases affecting the potato. It has been reported as damaging potatoes throughout the various potato-growing areas of the United States and Alaska. Alberts (1) reported rhizoctonosis as one of the most serious diseases of the potato in Alaska. Gratz (7) reported it from Florida. Many investigators have reported the disease on stems and tubers throughout the more temperate zone.

The influence of such factors as temperature, soil moisture, and strains of the fungus have not been definitely settled. All investigators seem to agree that conditions favorable for stem infection differ in some respects from the optimum for the development of sclerotia on the tubers. Gratz (7) found that stem lesions were quite common in Maine but seldom a factor in Florida. Richards (15), reporting on his studies in Wisconsin, says "The results in general indicate that the occurrence of an average daily temperature of 21° C.—on the growing points during the first six weeks of growth may accurately govern the damage done to the crop by the fungus in any one season. Richards (14) again found that the fungus may be parasitic through a wide range of temperatures and that serious damage to growing point and tissue occurred at 18° C.

Edson and Shapovalov (6) found that several species of Rhizoctonia are capable of attacking the underground portions of the potato stem. Rolfs (16) concluded that a high temperature and abundant moisture were essential for the rapid development of the fungus. He correlates the seriousness of the disease in Colorado with the practice of excessive irrigation during periods of high soil temperature. Peltier (12) concluded that a high temperature, 88° F., and moisture content of the soil above or below that most favorable for plant development, determined to a large degree, the virulence of strains. He evidently referred to air temperature, since no data on soil temperature were given with reference to the above conclusion. Balls (2), working in Egypt, found that low temperature favored the devel-

opment of the rhizoctonia fungus on cotton and that in some cases the plant might not become infected even when growing in a soil heavily infested with the active mycelium.

The greatest amount of work has been done on the stem-lesion phase of the disease. The sclerotial stage found on the tubers, apparently, has been considered less important than the stem-infection injury, and several investigators have studied the factors influencing that type of injury. Duggar (5) reported 2 types of infection, the common sclerotial type and the scab type. Rolfs (17) described the cracked, scabby type as damaging potato tubers in Colorado. Matsumoto (10) discussed strains of the rhizoctonia organism on many hosts. He reported 6 different strains and that all showed increased growth on the acid side of neutrality. Ramsey (13) described a form of rhizoctonia injury resembling scab, in some respects. Rosenbaum and Shapovalov (18) showed quite conclusively that strains of the fungus exist and that certain strains are more pathogenic on potatoes than others.

Considerable work has been done on the control of rhizoctonosis of potatoes. Most of the control measures have been directed towards the killing of the tuber borne sclerotia. Schultz, Gratz, and Bonde (19) found that in Maine seed-potato treatment with most of the materials commonly used for that purpose reduced stem infection and tuber-borne sclerotia. They found that soaking tubers in a solution of corrosive sublimate was the most effective treatment. Clayton (3), working in the Long Island area of New York State, found that organic mercury compounds were less effective against Rhizoctonia than was corrosive sublimate. He also concluded that seed treatment was not profitable for the Long Island grower, since soil infection was high. MacMillan and Christensen (9) found mercuric chloride effective in the control of rhizoctonosis in the form of a commercial gain as expressed in yield. McKay (8) found that the 1½-hour mercuric chloride treatment was superior to the half-hour treatment and that formaldehyde was ineffective. Stakman and Tolaas (20) found seed selection and seed treatment with corrosive sublimate and formaldehyde effective against Rhizoctonia. Their results showed corrosive sublimate more effective than formaldehyde. Melhus and Gilman (11) described in detail the hot-formaldehyde method of treating seed potatoes for control of rhizoctonosis and scab. This method is popular in the Greeley area. Dana (4), working in Washington, made a comprehensive study of Rhizoctonia. He took up several phases of the disease and recommended a combination of seed treatment and certain cultural practices for its most effective control.

GENERAL METHODS

The period covered by the studies here presented includes the growing seasons of 1930–31–32. The region covered includes several potato-growing

areas located in various sections of Colorado. Experimental data, for the most part, were taken from experiments conducted on the Colorado Potato Experiment Station at Greeley, Colorado. Observations relative to occurrence and severity of infection were made in the irrigated areas of both the plains and mountain sections. Control experiments included seed treatment and cultural practices as affecting the sclerotial stage of the disease on the tubers. The variety Bliss Triumph was used in all of the experiments.

The following experiments, for the most part, were conducted in the field. Laboratory and greenhouse work supplemented the field experiments. Soil temperatures were recorded by means of soil thermographs with the bulb placed at a depth of 5 inches. Soil moisture content was not determined. Controlled irrigation served to vary the amount of moisture received by each experimental plot. A plot, designated as heavy irrigation, received water in excess of normal plant needs. Another plot received light irrigation, which consisted of frequent applications of water but in quantities less than was necessary for maintenance of a vigorous growth of the plants.

Several types of seed tubers were used in the field experiments. Infected tubers consisted of those having a large number of rhizoctonia sclerotia. Clean tubers were free from any appreciable amount of sclerotial infection. In some cases the infected tubers were planted without treatment to insure the presence of inoculum. In others the infected seed tubers were treated, the object being to test the efficiency of several seed disinfectants. Tubers were considered free from the fungus when no sclerotia were visible to the naked eye. In several experiments this type of tuber was treated to insure complete freedom from the fungus.

In estimating the amount of disease present on stems, each stem was cultured and the organism identified. The amount of infection on tubers was determined by careful examination of each one from an experimental unit.

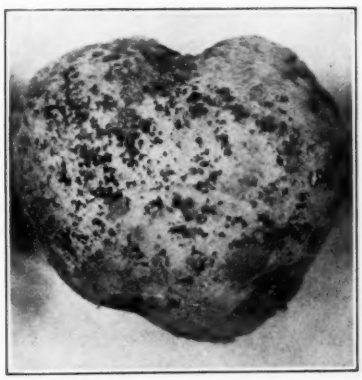


FIG. 1. Sclerotia on tuber. Medium heavy infection.

Those showing an appreciable number of sclerotia were considered infected. Figure 1 shows typical medium heavy sclerotial tuber infection.

RELATION OF SOIL TEMPERATURE TO STEM INFECTION

The yield of early potatoes in the Greeley, Colorado, section, usually is lower than that of those planted as a late or main crop. A higher market price usually compensates for the smaller yield; consequently, growers plant as early as possible. Many fields are planted during the first week of April, so these young plants often are injured by frost.

Stem lesions caused by Rhizoctonia (*Corticium vagum*) often do considerable damage to this early crop. Low soil temperature and rhizoctonia damage on the stems appear to be closely related. In the higher altitude of the mountain valleys, where low soil temperatures continue through most of the growing season, stem lesions are considered as one of the limiting factors to potato culture. Ninety per cent infection has been noted in several mountain valleys and also in the early potato section near Greeley. The stems often become infected before the young shoots are above ground, and this girdling often kills the sprouts and causes poor stands. The more common type of injury is the lesion infection, where only a part of the stem is injured. The plant does not die out but its vitality is reduced. As the season progresses and the soil becomes warmer these plants often recover and produce a fair yield.

An experiment designed to determine the effect of soil temperatures on rhizoctonia infection on the stems of potatoes, was conducted in the early crop section near Gilcrest, Colorado, in 1932. Sixty seed pieces bearing a large number of sclerotia were planted each week for six weeks, beginning April 1. Each week as the infected seed was planted, a check plot of the same number of hills was planted with seed free from sclerotia. Soil-temperature data were taken at a depth of 5 inches. Accurate soil-moisture records were not taken.

Six weeks after planting each lot was dug and the stems were examined for rhizoctonia infection. Those stems showing infection were cultured, the organism isolated and identified. Table 1 shows the results of this experiment. Data from the check plots were not included in the table, since the amount of infection was light and not uniform. The greatest amount of infection occurred on the plot planted April 8. Of the 60 stems examined, 44 showed rhizoctonia infection. Of these, 31 contained lesions covering large areas of the stem. The percentage of infection was high on the plot planted April 1, but the type of injury was less severe than on the plot planted April 8. Tubers planted after May 1, became only slightly infected. Only 8 of the 60 plants showed infection and none showed presence of lesions covering a large area of the stem. Figure 2 shows stem with typical lesions.

TABLE 1.—*Effect of soil temperature on infection of potato stems by Rhizoctonia. Records made 44 days after planting. Greeley, Colorado, 1932*

Number of plants	Date planted	Date stems were examined	Number of stems showing infection	Number of stems showing severe infection	Mean min. soil temperature 44 day period	Mean maximum temperature 44 day period	Mean temperature 44 day period
					°*F.*	°*F.*	°*F.*
60	April 1	May 15	32	16	39.5	61.0	50.2
60	April 8	May 22	44	31	41.8	63.5	52.6
60	April 15	May 29	37	23	44.3	65.7	55.0
60	April 22	June 5	26	2	45.4	67.5	56.4
60	April 29	June 12	20	4	48.2	72.3	60.2
60	May 7	June 20	8	0	55.0	73.5	64.3

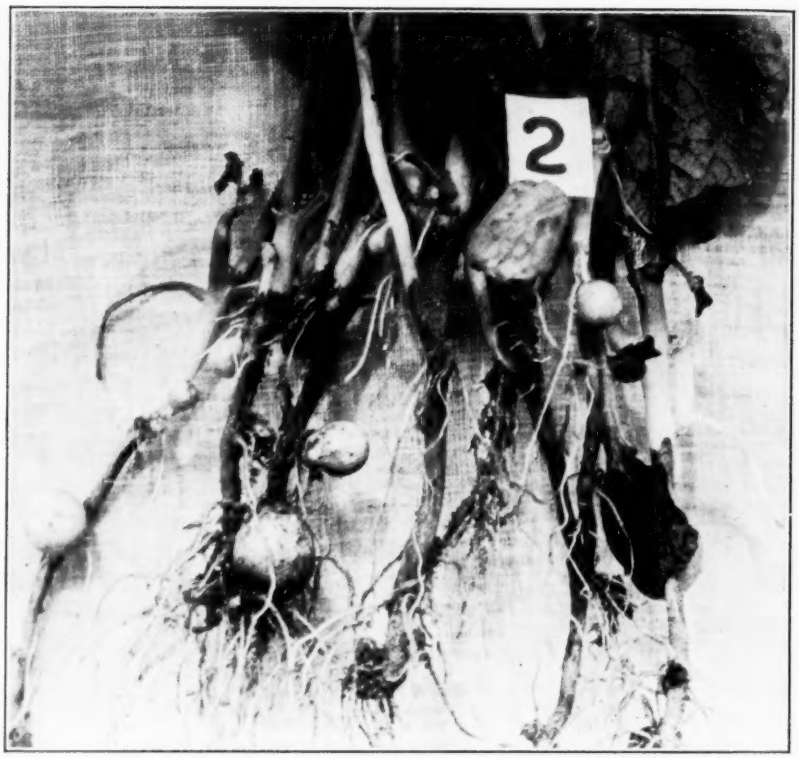


FIG. 2. Typical stem lesions caused by Rhizoctonia.

The average minimum soil temperature for the period April 8 to May 22 was 41° F., and the average maximum was 63.5° with a mean of 52.6° for the 44-day period. The most severe infection occurred during this 44-day period, indicating that the optimum soil temperature for stem infection is between 41.8° F. and 63.5°. These results agree with field observations that the most severe stem infection occurs early in the season when soil temperatures are quite low. Although the fungus is parasitic throughout a wide range of temperatures, as stated by Richards (15), the lower soil temperatures of the early growing season and the higher mountain valleys no doubt favor the development of the stem-lesion phase of the disease.

THE EFFECT OF LIGHT, NORMAL, AND HEAVY IRRIGATION ON THE DEVELOPMENT OF SCLEROTIA ON TUBERS

Preliminary experiments and observations made in several commercial fields indicated that moisture in excess of the normal needs of potato plants

is an important factor in the development of the sclerotial stage of *Corticium vagum*. When potatoes were given an excess of moisture throughout the growing season, many of the tubers were found to be heavily infected with sclerotia. An experiment to test the effect of excess irrigation water on the infection and development of sclerotia was started during the season of 1931. Both clean and infected tubers were used. The amount of irrigation water was varied by the number and amount of applications each lot received. Temperature data were recorded by soil thermographs with the bulbs placed at a depth of five inches. Each plot consisted of a row containing $\frac{1}{16}$ acre. All tubers from a plot were examined and the percentage of infected ones computed on the basis of the total weight of tubers from the plot.

In table 2, are shown the kind of seed tubers used, the amount of water applied, the percentage of infected tubers harvested, and the soil temperature for the different plots. Tubers containing sclerotia were used in several of the units to insure the presence of inoculum. The rows receiving heavy irrigation were watered as often as was necessary to keep the soil nearly saturated. The units receiving normal irrigation were given sufficient water to keep the plants in an active growing state. Light irrigation consisted of fewer light applications of water than was considered good practice. Plots with no irrigation received only such water as fell in the form or rain, which was not of sufficient quantity to allow normal plant growth.

The degree of infection in the different plots varied considerably. Any tuber having on it sufficient sclerotia to be readily visible was considered infected. Many of the tubers were so heavily infected as to be discriminated against by buyers.

The highest percentage of rhizoctonia infection was found on the tubers from the plot receiving heavy irrigation. The season's mean soil temperature was lowest in this plot. Table 2, shows the soil-temperature data taken in the plots receiving light, normal, and heavy irrigation. The percentage of tuber infection corresponded closely with the amounts of water applied and the correspondingly low mean soil temperature. That the soil temperatures varied but little between the three plots representing the different types of irrigation, leads to the conclusion that *Corticium vagum* is less sensitive to temperature variations than to the moisture content of the soil.

The presence of inoculum on the seed pieces increased the amount of infection on the plots receiving heavy and normal irrigation. There was but little increase from this cause in the plots grown under light or no irrigation. The results, in a measure, agree with those obtained by Rolfs (16) who found that abundant moisture and high temperature favored the

TABLE 2.—*Relation of irrigation to the development of sclerotia on tubers. Greeley, Colorado. 1931*

Condition of seed	Type of irrigation	Total weight of tubers	Infection	Mean max. soil temp.	Mean min. soil temp.	Season mean soil temp.
		Pounds	*per cent*	°*F.*	°*F.*	°*F.*
Infected	Heavy	229	30.4	82.1	65.7	73.9
Infected	Normal	308	9.5	79.4	68.1	73.7
Infected	Light	226	3.0	82.6	67.7	75.1
Infected	None	93	3.0	No data	No data	No data
Clean[a]	Heavy	310	13.0	" "	" "	" "
Clean	Normal	306	3.5	" "	" "	" "
Clean	Light	197	3.0	" "	" "	" "
Clean	None	83	2.5	" "	" "	" "

[a] Seed treated with mercuric chloride, 1–1000, 1½ hours.

development of the fungus. Especially did he find that abundant moisture is essential for optimum development of sclerotia.

EFFECT OF HEAVY IRRIGATION NEAR THE END OF THE GROWING SEASON ON THE DEVELOPMENT OF SCLEROTIA ON TUBERS

Observations in rhizoctonia-infested fields have shown that sclerotial infection on tubers is always most serious in plots that had received large quantities of irrigation water during the last 30 days of the growing period. This practice of heavy, late irrigation is common in the Greeley area.

An experiment to determine the effect of heavy, late-season irrigation on the development of sclerotia on tubers was started in the season of 1931 and carried through that of 1932. Both infected and clean tubers were planted in 2 plots of 4 rows each. Each row contained $\frac{1}{16}$ A. Both plots received normal irrigation until approximately 30 days before harvest, when the heavy-irrigation plot was given several copious applications of water. Only light applications were given to the normally irrigated plot. In table 3 the results of this experiment for the 1931 season are given. Those of the 1932 season were essentially the same and are not given.

The tubers from the heavily irrigated plot were covered with soil that was removed with difficulty, because of large numbers of sclerotia. Those from the normally irrigated plot were clean and essentially free from sclerotia. Each tuber from both plots was carefully examined for sclerotia and the percentage of infection computed on the basis of total weight of tubers.

The number of sclerotia on the tubers varied, but when present they were in sufficient numbers to be readily noticed. Many tubers from plot 2 were almost unsalable because of many sclerotia. The infected tubers produced tubers containing the highest percentage of sclerotial infection. Each plot planted with clean tubers produced cleaner ones. Yield was affected but little by the two types of irrigation, the normally irrigated plot produced a slightly higher yield.

There is but little difference in the mean soil temperature for the last 30 days growth period of the two plots. The lower mean temperature existed in the heavily irrigated plot, but is of such small difference that it seems doubtful if soil temperature was a factor in the results of the experiment.

SEED TREATMENT AS A CONTROL OF TUBER INFECTION

Several seed treatments have been recommended by different investigators for control of stem infection caused by *Corticium vagum*. Schultz *et al* (19) found that seed treatment with most of the materials commonly used reduced both stem and tuber infection. Clayton (3) found mercuric

TABLE 3.—*Effect of heavy late-season irrigation on the development of sclerotia on tubers. Greeley, Colo., 1931*

Row	Plot 1					Plot 2				
	Normal irrigation					Heavy late irrigation				
	Condition of seed	Number of hills	Yield in pounds	Percentage of tubers with sclerotia	Mean soil temp. last 30 days	Condition of seed	Number of hills	Yield in pounds	Percentage of tubers with sclerotia	Mean soil temp. last 30 days
					°F.					°F.
1	Clean	190	322	3.1	71.3	Clean	190	281	14.2	67.2
2	Infected	187	281	10.2	70.6	Infected	190	259	44.3	67.3
3	Clean	190	304	3.4	69.9	Clean	187	287	10.0	67.0
4	Infected	186	324	14.0	70.6	Infected	189	291	35.1	68.5

chloride superior to organic mercury compounds for control of rhizoctonosis. He found also that when soil infestation was high seed-tuber treatment was of little value in the Long Island, New York, area.

In Colorado nearly all potatoes are grown under irrigation, and often the tubers are heavily infected with Rhizoctonia. Much locally grown seed is used and the problem of tuber treatment becomes an important factor for the control of stem lesions in the early-crop area and for the reduction of tuber infection in the late-crop sections.

Mercuric chloride, formaldehyde, and several of the commercial organic mercury compounds are used as seed disinfectants. Often the treatment must serve to control both rhizoctonosis and common scab, and mercuric chloride is most commonly used.

An experiment designed to test the value of several organic mercury compounds, mercuric chloride, and formaldehyde for the control of sclerotia on tubers was started in the season of 1931. The following experiments were conducted in the late-crop plots, where stem infection seldom is a problem.

The organic mercury compounds used included several supplied by the Bayer Co. and were known only by numbers. No name was supplied with the materials. The dilutions used were suggested by the manufacturers. Seed designated as clean was apparently free from sclerotia, since none were visible to the naked eye.

Two types of mercuric chloride treatment were used, the common 1–1000 treatment for 1½ hours, and the acidulated mercuric chloride solution. In the latter 6 ounces of mercuric chloride were dissolved in 1¾ pints of 31 per cent hydrochloric acid and added to 22 gallons of water. The whole tuber was allowed to remain in this solution for 3 minutes, then drained and dried. In the hot-formaldehyde treatment the cut tuber was immersed for 2 minutes in a solution containing 1 pint of commercial formaldehyde in 15 gallons of water held at 122° F., then removed and allowed to dry. In table 4 are given the results obtained from the several types of seed treatment.

Where sclerotia were present on the seed, all treatments were of value. The highest yield was from apparently clean seed treated with mercuric chloride. Untreated sclerotia-bearing seed produced a crop 47 per cent of which was infected, the infection varying from severe to light. Many of the tubers were so badly affected with sclerotia as to be discriminated against by buyers. Untreated clean seed produced a crop relatively free from infection.

Hot formaldehyde proved to be of less value than mercury chloride for killing the sclerotia on the seed tubers as indicated by the relatively

TABLE 4.—*Results of seed-potato treatment for control of sclerotia on tubers. Greeley, Colo., 1932*

Condition of seed planted	Treatment	Yield bu. per acre	Percentage of tubers bearing sclerotia[a]
Clean[a]	Mercuric chloride 1–1000 1½ hrs.	329.00	3.6
Clean[b]	Untreated	318.00	9.4
Sclerotia on surface	Mercuric chloride 1–1000 1½ hrs.	301.00	7.1
Sclerotia on surface	Untreated	294.50	47.0
Sclerotia on surface	Acid mercuric chloride. 3 min.	270.00	17.0
Sclerotia on surface	Hot formaldehyde 2 min. 122° F.	284.00	13.3
Sclerotia on surface	Bayer organic mercury #694	321.00	14.0
Sclerotia on surface	Bayer organic mercury #738[c]	234.00	11.7
Sclerotia on surface	Bayer organic mercury #664	299.10	15.5
Sclerotia on surface	Semesan Bel.	286.50	16.1

[a] By weight.
[b] Apparently clean seed.
[c] Poor stand. Seed piece injury by treatment.

high percentage of infected tubers. The 1–1000 mercuric chloride treatment was more effective than the acid mercuric chloride.

The 4 organic mercury compounds did not control sclerotial infection as effectively as did mercuric chloride. Bayer No. 694, increased the yield over the mercuric chloride and formaldehyde treatments, but the percentage of infected tubers was higher. The increased yields in one of the organic mercury plots was partly due to a better stand.

These results would indicate light soil infection by *Corticium vagum*, and also that most of the infection on the tubers resulted from infected seed.

SUMMARY

Rhizoctonosis of potatoes, caused by *Corticium vagum*, is common in the irrigated areas of the Greeley, Colorado, section. The stem-infection phase

of the disease is most common on the early-crop potatoes grown in a cool soil. Damage to the plants through reduced vitality caused by stem lesions may be the common type of injury in the early-crop areas. Tuber infection is rarely a factor in the early crop.

Experiments conducted near Gilcrest, Colorado, in the early-crop area, showed serious stem infection when the crop was planted early in cold soils. Plantings made late in the season are relatively free from stem infection.

Excess moisture and low soil temperatures have been observed to be coincident with heavy sclerotial infection on tubers. Experiments indicate that more severe tuber infection occurs under conditions of heavy irrigation. Lowered soil temperature caused by excessive irrigation apparently is not so important as is moisture in the development of sclerotia on tubers.

Irrigation water, applied in large quantities late in the season, increased the number of sclerotia on tubers. Careful handling of irrigation water towards the close of the growing season is suggested as a control measure for tuber infection.

Seed-tuber treatment is of value in reducing the amount of rhizoctonia infection on tubers. Mercuric chloride was found to be of more value than formaldehyde or any of the organic mercury compounds.

A combination of seed treatment and careful handling of irrigation water during the last few weeks of tuber growth is suggested for the control of tuber infection by rhizoctonia.

GREELEY, COLORADO.

LITERATURE CITED

(1) ALBERTS, H. W. The potato in Alaska. Alaska Agr. Exp. Stas. Bul. 9. 1931.

(2) BALLS, W. LAWRENCE. The physiology of a simple parasite. Yearbook Khediv. Agr. Soc. **1905**: 171–195. 1906.

(3) CLAYTON, E. E. Potato seed treatment on Long Island with special reference to the organic mercury instant dips. New York State Agr. Exp. Sta. Bul. 564. 1929.

(4) DANA, B. F. The Rhizoctonia disease of potatoes. Wash. Agr. Exp. Sta. Bul. (Tech. Paper) 191. 1925.

(5) DUGGAR, B. M. *Rhizoctonia crocorum* (Pers.) D.C. and *R. solani* Kühn (*Corticium vagum* B & C.) with notes on other species. Ann. Missouri Bot. Gard. **2**: 403–458. 1915.

(6) EDSON, H. A., and M. SHAPOVALOV. Potato stem lesions. Jour. Agr. Res. **14**: 213–220. 1918.

(7) GRATZ, L. O. Disease and climate as pertaining to Florida and Maine potato sections. Phytopath. **20**: 267–288. 1930.

(8) MCKAY, M. B. Potato diseases in Oregon and their control. Oregon Agr. Exp. Sta. Circ. 24. 1922.

(9) MACMILLAN, H. G., and AXELL CHRISTENSEN. A study of potato seed treatment for Rhizoctonia control. Wyoming Agr. Exp. Sta. Bul. 152. 1927.

(10) MATSUMOTO, TAKAHASHI. Studies in physiology of the fungi. XII. Physiologic specialization in *Rhizoctonia solani* Kühn. Ann. Missouri Bot. Garden **8**: 1–62. 1921.

(11) MELHUS, I. E., and J. C. GILMAN. An improved method of potato seed treatment. Iowa Agr. Exp. Sta. Circ. 57. 1919.

(12) PELTIER, GEO. L. Parasitic Rhizoctonias in America. Illinois Agr. Exp. Sta. Bul. 189. 283–390. 1916.

(13) RAMSEY, G. B. A form of potato disease produced by Rhizoctonia. Jour. Agr. Res. **9**: 421–426. 1917.

(14) RICHARDS, B. L. Pathogenicity of *Corticium vagum* on the potato as affected by soil temperature. Jour. Agr. Res. **21**: 459–482. 1921.

(15) ————————. Further studies on the pathogenicity of *Corticium vagum* on the potato as affected by soil temperature. Jour. Agr. Res. **23**: 761–770. 1923.

(16) ROLFS, F. M. Potato failures. Colorado Agr. Exp. Sta. Bul. 70: 1–20. 1902.

(17) ————————. Potato failures. Colorado Agr. Exp. Sta. Bul. 91: 1–33. 1904.

(18) ROSENBAUM, J., and M. SHAPOVALOV. A new strain of *Rhizoctonia solani* on the potato. Jour. Agr. Res. **9**: 413–419. 1917.

(19) SCHULTZ, E. S., L. O. GRATZ, and REINER BONDE. Effect of seed potato treatment on yield and Rhizoctonia in northeastern Maine from 1925 to 1928. Phytopath. **20**: 47–64. 1930.

(20) STAKMAN, E. C., and A. G. TOLAAS. Potato diseases and their control. Minn. Agr. Exp. Sta. Bul. 158. 1916.

THE NATURE OF INHIBITION BETWEEN CERTAIN FUNGI PARASITIC ON CITRUS[1]

JAIME GUISCAFRÉ ARRILLAGA

(Accepted for publication October 25, 1934)

INTRODUCTION

The importance of investigations on mixed cultures of organisms was pointed out concisely by Fawcett (2) in his statement " . . . since Koch and others perfected the pure-culture methods . . . students have been thoroughly imbued with the idea that cultures must be pure for a single organism. . . . The insistence on purity of cultures was, indeed, a very important condition for preliminary work for the study of the part played by a single organism in the absence of all others. . . . We have now come to a stage requiring synthesis as well as analysis, integration as well as differentiation. . . . Nature does not work with pure cultures alone but most frequently with associations."

According to the literature, interesting behaviors of mixed cultures of organisms have been reported, based both on accidental observations and on purposely made investigations.

In general the studies of mixed cultures have shown two different types of behavior; in some cases the culturing of one organism in the presence of another has resulted in the production of reproductive bodies; in other cases the associated culture of organisms has led to a partial, strong, or mutual inhibition in the rate of growth and size of the colonies of the organisms involved. Striking cases also have been shown where the presence of another organism greatly accelerated the growth or action of another or where the mutual action was much increased.

In connection with the first type of behavior (stimulation to formation of reproductive bodies), Molliard (13); Heald and Pool (7); Sartory (16) and (17); and McCormick (11), have reported some interesting cases.

In 1924, Porter (14) made an extensive study of combinations of fungi and of fungi with bacteria, and, after observing their behavior in culture, described 5 different types of inhibition.

Machacek (12) in his studies of mixed cultures on fruits, found out that *Penicillium expansum* and *Cladosporium fulvum* inhibited completely the growth of *Sclerotinia fructicola* and *Botrytis* sp., respectively.

Fawcett and Lee (4, p. 37–38) report that combined inoculations with *Phytophthora citrophthora* and *Fusarium* sp. on citrus trees, produced

[1] From a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science, published with the approval of the Dean of the Graduate School, Pennsylvania State College.

larger lesions and the decay proceeded faster and became more severe than inoculations with *P. citrophthora* alone.

Savastano and Fawcett (18) conducted a series of experiments with mixed cultures of fungi on fruits, studying especially the effect of temperature on the behavior of the combinations and they found that temperature had a decidedly selective effect in many cases, enabling one organism in a mixture to dominate the others in the production of decay.

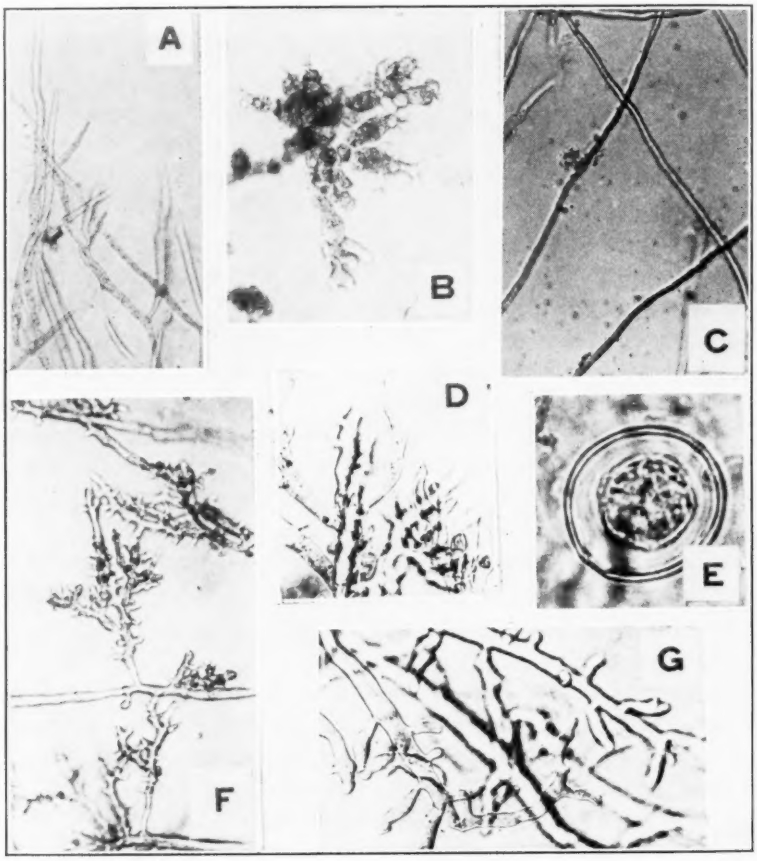


FIG. 1. A. Normal mycelium of *P. parasitica* when growing alone on potato-dextrose agar. B. Mycelium from the edge of a colony of *P. parasitica* growing within 3 mm. of a colony of *Diaporthe citri*, showing abnormal branching. C. Normal mycelium of *P. citrophthora* when growing alone on potato-dextrose agar. D. Effects of the same filtrate sterilized in the autoclave for 20 minutes at 20 lbs. pressure and 110° C., on *P. citrophthora*. E. Oogonia-like body of *P. citrophthora* as observed in liquid cultures and oranges. F. Mycelium from the edge of a colony of *P. citrophthora* growing within 3 mm. of a colony of *Diaporthe citri*, showing abnormal branching. G. Effect of the filtrate obtained from a 20-day-old culture of *Diaporthe citri* in Czapek's liquid media, on the mycelium of *P. citrophthora*.

Vasudeva (21) observed that the presence of *Botrytis allii* in the inoculum of *Monilia fructigena* reduced strikingly the pathogenicity of the latter.

Gioelli (5) reported that the presence of *Penicillium digitatum* completely checked the growth of *P. italicum* in fruit rots.

Reid (15) did some work on the bacterial inhibitory properties of the mold, *Penicillium notatum,* especially upon the nature of the filtrate containing the staling products of the mold.

Concerning the substance responsible for stimulation or inhibition in such cases little is definitely known. Some believe the action purely enzymatic, or a question of availability of nutrients; others think it due to an osmotic relationship or merely a matter of toxicity.

With these views in mind, the writer conducted a series of experiments with mixed cultures of fungi in an endeavor to throw more light on the subject.

MATERIALS AND METHODS

For the studies, 12 species of fungi, commonly associated with citrus fruits were used: *Penicillium digitatum* Sacc.: *P. italicum* Wehmer; *Colletotrichum gloeosporioides* Penz.; *Alternaria citri* Ellis and Pierce; *Diplodia natalensis* Evans; *Diaporthe citri* Wolf; *Mucor spinescens* Lendn.; *Sclerotium rolfsii* Sacc.; *Botrytis cinerea* Pers.; *Sclerotinia libertiana* Fckl.; *Phytophthora parasitica* Dastur; and *P. citrophthora* (Sm. & Sm.) Leonian.

The 12 fungi were first grown in Petri dishes in pairs. In these tests, all possible combinations of two were set up. Potato dextrose agar was used and inoculations were made with bits of mycelium from pure cultures, the two fungi being placed about 3 cm. apart. Similar combinations were set up on potato-dextrose agar in van Tieghem cells for more careful observation.

Of the combinations tried, the most interesting were: *Diaporthe citri* × *Phytophthora parasitica* and *D. citri* × *P. citrophthora.* In these cases not only was the shape and size of the colonies affected but, also, striking morphological changes occurred in the mycelium of the *P.* spp. (Figs. 1, A, B, C, F, and 2). These 2 combinations, therefore, were made the object of further studies.

Experiments were designed to determine, (a) whether the inhibitory substance produced by *Diaporthe citri* was given off in the form of a gas or radiation, (b) effect of the hydrogen-ion concentration in the zone of inhibition, (c) the effects of filtrates containing the metabolic products of *D. citri,* upon the mycelium of the species of Phytophthora, (d) effects of the extracts made from the living mycelium of *D. citri* on the Phytophthora, (e) the behavior of the combinations in liquid cultures using Czapek's and a modification of Coons' (1) liquid medium.

Experiments also were conducted by making inoculations into grape-

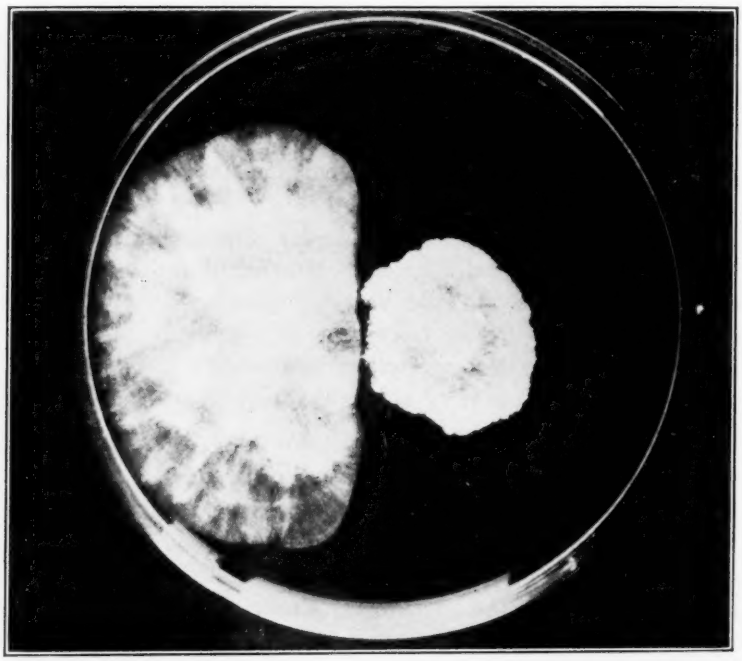


FIG. 2. Colonies of *Diaporthe citri* (right) and *P. citrophthora* (left) after four days of growth on potato-dextrose agar.

fruits, oranges and lemons, to determine whether or not the results already obtained in artificial media could be obtained in fruits.

All the experiments were conducted at room temperatures of 25 to 28° C.

RESULTS

It was found in the preliminary tests, that the various combinations could be grouped into 7 types. Porter (14) described 5 types in similar cases. Four of these were recognized, as well as 3 additional ones not mentioned by him. These 3 types may be described as follows: (a) colonies whose growth stops in front of each other leaving in between a conspicuous straight or somewhat curved free zone, both colonies are checked in growth, (b) a colony that, when approaching that of a different fungus, grows under and then reaches the surface again and continues to grow normally, (one of the colonies is checked) and (c) colonies which when approaching each other are mutually checked in rate of growth, and in one of which striking morphological changes occur in the mycelia. To this last type belong the combinations of *Diaporthe citri* with either of the species of Phytophthora.

The interesting cases of inhibition observed in the combinations of *Diaporthe citri* with either of the species of Phytophthora may be described

as follows: Whenever the edges of the colonies of either *Phytophthora parasitica* or *P. citrophthora* came within 3 mm. of the edge of a colony of *D. citri*, the mycelium of the Phytophthora became strikingly changed, abnormally branched, like a witches' broom and having unusual tendencies to fruiting. (Fig. 1, B and F). On the other hand, colonies of either *P. parasitica* or *P. citrophthora* growing singly in Petri dishes retained their normal shape and growth indefinitely. Also, the colonies of both of the species of Phytophthora, when examined on the edges opposite to the colony of *D. citri*, presented a completely normal appearance.

The fact that inhibition occurred when the colonies were separated by a distance of 3 mm. or more, and the characteristic way in which they stopped in front of each other, made it of interest to determine whether the inhibitory substance was volatile or simply diffusible through the medium (Fig. 2).

For determining the diffusibility of the substance produced by *Diaporthe citri* and its action upon species of Phytophthora, liquid cultures and filtration and extraction experiments were conducted. For determining whether the substance was volatile, a special 2-layer Petri dish was used. The method consisted in the preparation of Petri dishes by pouring potato-dextrose agar in both the dish and its cover. The dishes thus prepared were inoculated with combinations of *D. citri* and *P. parasitica* and *D. citri* and *P. citrophthora*. But one fungus was planted in the dish, while the other was planted in the cover around the center, but so placing the transfers that the 2 resulting colonies finally would be separated by an air space of 3 mm. and a lateral distance of 3 cm. To avoid the possibility of any excretion or secretion of the mycelium of *Diaporthe citri* falling upon the mycelium of either species of Phytophthora, the Petri dishes were maintained constantly in a vertical position.

Observation of all cultures thus prepared showed that both *Phytophthora parasitica* and *P. citrophthora* developed radially to full size and no morphological changes were noticed in their mycelium, proving that if any gas or radiation was given off by *Diaporthe citri* it did not affect the mycelium of Phytophthora.

To determine whether hydrogen-ion concentration was a factor in the inhibition, pH studies were conducted. The pH was determined by the use of the potentiometer. For the determinations, a narrow strip of agar was removed from the zone immediately surrounding the colonies.

The figures obtained in the zones of inhibition seem not to differ enough from those of the margins of normal colonies to warrant any assumption that acidity plays a very important inhibitory rôle.

The results thus far obtained indicated the production of a soluble, non-volatile substance by *Diaporthe citri* as the principal factor in the inhibition of Phytophthora.

TABLE 1.—*The relation of pH to inhibition betweeen Diaporthe citri and Phytophthora species*

Origin of sample for pH determination	Ave. pH
Zone around colony of *Phytophthora parasitica* growing alone	6.21
Zone around colony of *P. citrophora* growing alone	5.74
Zone around colony of *Diaporthe citri* growing alone	5.87
Zone of inhibition between colonies of *D. citri* and *P. parasitica*	5.38–6.20
Zone of inhibition between colonies of *D. citri* and *P. citrophora*	5.24–5.38
Potato dextrose media alone	5.6

The greater possibilities for the diffusion of such a substance in a liquid medium led to the following experiments. The 2 liquid media used were Czapek's solution and an arbitrary modification of Coons' solution (1).

Both Petri dishes and test tubes were used. The Petri dishes, containing 15 cc. of medium, were inoculated by floating bits of mycelium of the combination *Diaporthe citri* and *Phytophthora parasitica* and of *D. citri* and *P. citrophthora* on the liquid; the inocula were placed always at a distance of 3 cm. from each other and in the center of the Petri dishes. The test tubes also were inoculated with both fungi. Controls were run with single inoculations of *P. parasitica* and *P. citrophthora.*

Observations were recorded at intervals of 5 to 7 days for a period of 2 weeks. The following table summarizes the results in Czapek's liquid medium in Petri dishes.

TABLE 2.—*Reaction of Phytophthora parasitica and P. citrophthora with Diaporthe citri in Czapek's and in Coons' solution in Petri dishes*

Fungi used	Culture solution	Observations			
		First week	Second week	Third week	Fourth week
D. citri × *P. parasitica*	Czapek's	x[a]	xxzz	xxzzo	xxzc
P. parasitica (alone)	"	normal	normal	z	zc
D. citri × *P. citrophthora*	"	x	xxzz	xxzzo	xxzc
P. citrophthora (alone)	"	normal	normal	z	zc
D. citri × *P. citrophthora*	Coons'	x	xxzo	xxzzoo	xxzco
P. citrophthora (alone)	"	normal	normal	z	zc

[a] The following legend is used for tables 1 and 2. x—mycelium slightly affected; xx—mycelium strongly affected; o—few oogonia-like bodies; oo—many oogonia-like bodies; c—few chlamydospores; cc—many chlamydospores; z—few zoosporangia; zz—many zoosporangia.

In test tubes, the same results were obtained, but the reproductive structures were less abundantly produced.

The growth of both species of Phytophthora was not abundant; the mycelium spread thinly and less extensively than on the solid medium. In the presence of *Diaporthe citri,* the mycelium of both species of Phytophthora was characteristically affected and the reproductive bodies were always found more abundant in connection with the affected regions.

The growth of the mycelium of *Phytophthora citrophthora* in Coons' solution was very scanty, but the characteristic branching always was observed in the presence of *Diaporthe citri,* and the reproductive bodies were found associated with the affected regions.

It will be noticed in the preceding tables that the colonies of *Phytophthora parasitica* and *P. citrophthora* were not only inhibited and the morphology of the mycelium altered, but, also, that the formation of reproductive bodies, such as zoosporangia, oogonia, and chlamydospores, was stimulated.

THE OOGONIA-LIKE BODIES OF PHYTOPHTHORA CITROPHTHORA

Although these structures were not found in large numbers, all those observed had what appeared to be a distinct oosphere in the center, which distinguished them easily from the chlamydospores. Their size also was smaller; measurements of over 100 giving a range of 17 to 27 μ in diameter. When stained with eosin they became orange yellow in contrast to the reddened mycelium and the reproductive structures. Although the writer is not certain as to the presence of antheridia, in many cases lateral, dark, club-shape structures were seen closely attached to or independent of the oosphere. The chlamydospores in both Czapek's and Coons' media measured from 27 to 44 μ. The zoosporangia, although commonly lemon-shape, varied considerably in shape and size. Their average size in both liquid media was 43×25 μ. The measurements agree with those of Smith (19); Leonian and Geer (10); Guiscafré (6); and Tucker (20). (Fig. 1, E). It is believed that some metabolic product of *Diaporthe citri* is the principal factor in making *P. citrophthora* produce these reproductive structures and especially the bodies here interpreted as oogonia, which have been observed only in cultures where both fungi were present.

In the filtration and extraction experiments, filtrates containing the metabolic products of *Diaporthe citri* were obtained in the following way: test tubes containing Czapek's medium were inoculated with *Diaporthe citri.* Some of the resultant cultures were grown 10 days; others, 20 days. Additional tubes were inoculated with a combination of *D. citri* and *Phytophthora parasitica* and *D. citri* and *P. citrophthora.* These combinations were grown for 20 days. At the end of the alloted growth period, the cultures were filtered through bacteriological filters. The filtrates thus obtained were tested as follows:

(a) Petri dishes were fitted with thin diametrically placed strips of cardboard. One of the halves was filled with potato-dextrose agar, and when this agar was well solidified, the cardboard strips were removed. The other half was then filled with a mixture consisting of $\frac{2}{3}$ of one of the filtrates of *Diaporthe citri* and the other third of $1\frac{1}{2}$ per cent plain agar. After this agar was well solidified, *Phytophthora parasitica* and *P. citrophthora* were transferred to Petri dishes thus prepared. The inoculations were made near the center of each dish and $1\frac{1}{2}$ cm. from the dividing line between the 2 kinds of agar.

(b) By filling half of the Petri dishes with potato-dextrose agar in which the species of Phytophthora to be tested was planted and by placing in the other half one of the liquid filtrates.

(c) By making transfers of Phytophthora to tubes containing 5 cc. of the filtrates of *Diaporthe citri.*

Observations made in the 3 different tests after periods of 7 to 10 days showed that the 10-day-old filtrate containing the metabolic products of *Diaporthe citri* had no effect on the mycelia of the species of Phytophthora; that the 20-day-old filtrate produced the characteristic abnormal branching in the species of Phytophthora; and that the inhibitory substance was produced by *Diaporthe citri,* alone, as well as in the presence of the species of Phytophthora.

The filtrates were then subjected to different conditions and tested as described above, on *Phytophthora citrophthora* only.

(a) The filtrate from a 10-day-old culture of *Diaporthe citri* was heated at 50° C. for 18 hours. Result: No effect on the mycelium of *Phytophthora citrophthora.*

(b) The filtrate from a 20-day-old culture of *Diaporthe citri* was heated at 50° C. for 18 hours. Result: Produced the characteristic abnormal branching in the mycelium of *P. citrophthora.* (Fig. 1, G).

(c) The filtrates from 20-day-old cultures of *Diaporthe citri* were autoclaved for 20 minutes at 110° C. and at 20 lbs. pressure, or boiled at 110° C. for 20 minutes in the open. Result: The mycelium of *Phytophthora citrophthora* showed the characteristic abnormal branching when tested with the filtrates thus treated (Fig. 1, D).

These experiments showed that the older the filtrate, the greater the effects produced in the mycelium of *P. citrophthora;* that by heating the 10-day filtrates for 18 hours at 50° C. the inhibitory substance apparently was not sufficiently concentrated to produce any effects; and that the inhibitory substance is thermostable under the conditions indicated.

Extracts of the living mycelium of *Diaporthe citri* were secured by growing the fungus in potato-dextrose agar for 15 days, scraping the mycelium from the agar and grinding it with 15 cc. of sterilized distilled water in a

mortar. The extracts were then run through a bacteriological filter and the filtrate was tested against *Phytophthora citrophthora*. Observations made after 10 days showed almost no effect on the mycelium of Phytophthora. This was to be expected, since, no doubt the inhibitory substance produced by *Diaporthe citri* doubtless had already diffused into the agar, leaving within the mycelium quantities insufficient to have any effect on the mycelium of *P. citrophthora*. However, different results might have been obtained if the extraction had been made at different stages of growth of the fungus.

For the fruit experiments, grapefruits, oranges, and lemons were used. In every case these were selected with attention to uniformity of size, color, and quality. Before inoculation the fruits were first treated with corrosive sublimate (1:1000) and then thoroughly rinsed with sterilized distilled water. Following inoculation the fruits were kept in glass moist chambers at room temperature. The inoculations were performed by making a superficial semicircular wound with a steel needle and placing the inoculum with a platinum needle.

In the first experiment the inoculum of the 2 fungi was placed separately at 3 cm. apart on the periphery of the fruits. Some fruits also were inoculated with each fungus separately to serve as controls.

Observations made after 10 days showed that, although the areas rotted by each fungus could not be well delimited, the mycelium of the Phytophthora was characteristically affected and showed the same abnormal branching observed in Petri dishes and liquid cultures. On the other hand, the fruits inoculated only with Phytophthora showed a completely normal mycelium.

In a second experiment both fungi of each combination were inoculated in the same wound.

Again, the same results were obtained, but this time zoosporangia and a few of the oogonia-like bodies were found in connection with the affected mycelium of *Phytophthora citrophthora* in the fruits inoculated with the combination of *Diaporthe citri* and *P. citrophthora* on the same wound.

In a third experiment this observation was further corroborated by inoculating a large number of oranges. The orange was used because *Diaporthe citri* grew better in it than in either the lemon or grapefruit and because the reproductive structures observed in the preceding experiment were found only in the orange.

In this experiment the reproductive structures were again observed. The controls showed a quite normal mycelium and no reproductive bodies.

A fourth experiment was conducted by inoculating *Phytophthora citrophthora* in fruits in which previously 5 cc. of the filtrate obtained from a 20-day-old culture of *Diaporthe citri* had been injected hypodermically.

Observations after 10 days showed that the mycelium of *Phytophthora citrophthora* was affected when it came in contact with the region in which the filtrate had been injected, but no reproductive structures were observed. Controls similarly treated, except that 5 cc. of sterilized distilled water had been injected into the fruits, showed the mycelium of *P. citrophthora* to be completely normal.

Observations on the fruit were made by crushing bits of tissue from the rotted areas and staining them with eosin. In cases of doubt, isolations were made from the rotted areas to test the identity of the fungus.

In general, the fruit experiments corroborated the results already obtained with the same associations of fungi in Petri dishes.

DISCUSSION AND CONCLUSIONS

Although the writer did not work with particular associations of fungi known in nature, the general behavior of the combinations studied and especially, the striking conditions brought about by a few, have proved definitely that each of these fungi behaves differently when grown in combination with some other fungus.

Opinions differ as to the factor or condition responsible for such inhibitions between organisms. In general, the following explanations have been offered: that it is an enzymatic relationship; an osmotic relationship; an exhaustion of nutrients; a matter of toxicity.

In this work, no attempt was made to determine the chemical composition of the inhibitory substance; but the experiments conducted have demonstrated that the inhibition is not caused by volatile metabolic products of *Diaporthe citri;* that there is no correlation between the hydrogen-ion concentration created by the fungi involved in the zone of inhibition and the inhibition produced; that the substance produced by *Diaporthe citri* is diffusible and filtrable; that the concentration bears a definite relationship to the effects produced on the mycelium of *Phytophthora* spp.; that it is relatively thermostable; and that the substance is produced with constancy, since the same effects were produced in potato-dextrose agar, Czapek's agar, Czapek's and Coons' solution, and on fruits: grapefruits, oranges, and lemons.

The appearance of the mycelium of *Phytophthora citrophthora* was not exactly the same in all the media employed; but this was to be expected since other species of Phytophthora have been found to present variations according to the media in which are grown, (Leonian, 8).

Although zoosporangia and chlamydospores also appeared in liquid cultures and fruits inoculated with *Phytophthora citrophthora,* only, their production and especially that of oogonia is undoubtedly stimulated by the presence of *Diaporthe citri,* since zoosporangia and chlamydospores always

were found in greater numbers and the oogonia were found only in cultures or fruits containing the inocula of both fungi.

The oogonia-like bodies produced by *Phytophthora citrophthora* in association with *Diaporthe citri* are clearly distinguishable from the chlamydospores by size, color of staining reaction, and the presence of an oosphere. The apparent lack of antheridia constitutes no argument against the possible presence of oogonia, here for the first time reported for this species, since the same condition (9) has been found to exist in other species of the genus.

The term inhibition has been used throughout the work, especially in connection with the associations of *Diaporthe citri* and *Phytophthora citrophthora*, since, in all cases, the vegetative growth of the species of Phytophthora is markedly checked. It may seem improper to use such a term when the fungi inhibited formed reproductive structures. The writer, however, believes that the substance produced by *D. citri* actually inhibits the growth of *P. citrophthora* and that the fruiting is merely an attempt on the part of *P. citrophthora* to survive the undesirable conditions introduced by the presence of another fungus, in its environment, at the expense of the checked vegetative growth.

More information is needed, not only as to how the inhibitory substance operates—whether it produces its effects as a result of an osmotic disturbance, or because it is merely toxic to the fungus protoplasm—but, also, as to its chemical nature.

The name *Diaporthe citri* has been used in preference to that of *Phomopsis citri*, since Fawcett (3) found that both *Phomopsis citri* and *Phomopsis californica* had the same perfect stage to which Wolf had already given the name of *Diaporthe citri*.

SUMMARY

Some fungi behave differently when cultured alone than when cultured in association with other fungi.

When certain fungi are cultured in pairs, generally, an inhibition takes place; this may be partial, strong, total, or mutual, checking the rate of growth of one or both fungi and in extreme cases, affecting the morphology and physiology of the fungus inhibited. Cases of acceleration of growth also occur.

Of the 78 combinations tried, those of *Diaporthe citri* with either *Phytophthora parasitica* or *P. citrophthora*, were the most interesting ones, since the growth of the species of Phytophthora was not only checked but also striking morphological and physiological disturbances were produced in the mycelia.

Such factors as metabolic products of *Diaporthe citri* given off in the form of a gas or radiation; habit of growth of the mycelia of the fungi in

the inferior layers of agar; hydrogen-ion concentration produced in the zone of inhibition; and temperature are believed to bear no relation to the inhibition.

A chemical substance contained in the metabolic products of *Diaporthe citri* is believed to be the real cause of the inhibition. This substance was found to be diffusible and filtrable, relatively thermostable, of greater activity in high concentrations, and to be constantly produced by *Diaporthe citri* on a variety of different substrata, just as well in the presence as in the absence of the species of Phytophthora.

Diaporthe citri was found to be the main factor involved in making *Phytophthora citrophthora* form the reproductive structures and, especially, of what are believed to be oogonia.

The chemical substance involved in the inhibition cannot be an enzyme; otherwise it would have been destroyed by a temperature of 110° C.

The growth of the species of Phytophthora is inhibited by a substance produced by *Diaporthe citri*. The formation of reproductive structures in the affected mycelium of *Phytophthora citrophthora* seems to be an indirect response to the stimulus exerted by the presence of *D. citri* in its environment.

BOX 282, MAYAGUEZ, PUERTO RICO.

LITERATURE CITED

1. COONS, G. H. Factors involved in the growth and the pycnidium formation of *Plenodomus fuscomaculans*. Journ. Agr. Res. 5: 713–769. 1916.
2. FAWCETT, H. S. The importance of investigations on the effects of known mixtures of microorganisms. Phytopath. 21: 545–550. 1931.
3. ————. *Diaporthe citri* (Faw.) Wolf, the perfect stage of *Phomopsis citri* and *P. californica*. Phytopath. 22: 928. 1932.
4. ————, and H. A. LEE. Citrus diseases and their control. 582 pp. McGraw-Hill Book Co., Inc. 1926.
5. GIOELLI, F. Fenomeni di in "*Penicillium digitatum*" (Pers.) Sacc. e "*Penicillium italicum*" Weber in natura. (Nota preliminare). Riv. Pat. Veg. 22: 195–200. 1932.
6. GUISCAFRÉ, J. R. The brown rot fungus in Puerto Rico. Jour. Dept. Agr. Puerto Rico 16: 193–202. 1932.
7. HEALD, F. D., and POOL, W. VENUS. The influence of chemical stimulation upon the production of perithecia by *Melanospora pampeana* Speg. Nebr. Agr. Expt. Sta. Rept. 22: 129–134. 1909.
8. LEONIAN, L. H. Physiological studies on the genus Phytophthora. Amer. Jour. Bot. 12: 448–498. 1925.
9. ————, and H. L. GEER. Heterothallism in Phytophthora. Phytopath. 21: 941–955. 1931.
10. ————, and ————. Comparative value of Phytophthora sporangia obtained under standard conditions. Jour. Agr. Res. 39: 293–311. 1929.
11. MCCORMICK, F. A. Perithecia of *Thielavia basicola* Zopf in culture and the stimulation of their production by extracts from other fungi. Conn. Agr. Expt. Sta. Bull. 269: 539–554. 1925.

12. MACHACEK, J. E. Studies on the association of certain phytopathogens. McGill Univ. Tech. Bull. 7. 1928.
13. MOLLIARD, M. Rôle des bactéries dans la production des périthèces des Ascobolus. Compt. Rend. **136**: 899–901. 1903.
14. PORTER, C. L. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Amer. Jour. Bot. **11**: 168–188. 1924.
15. REID, G. Some observations on bacterial inhibitory properties of a mold. Jour. of Bact. **29**: 215–221. 1935.
16. SARTORY, A. De l'influence d'une bactérie sur le production des périthèces chez un Aspergillus. Compt. Rend. Soc. Biol. Paris **79**: 174–175. 1916.
17. ————————. Production de périthèces chez un Aspergillus sous l'influence d'une bactérie. Compt. Rend. Soc. Biol. Paris **83**: 1113–1114. 1920.
18. SAVASTANO, G., and H. S. FAWCETT. A study of decay in citrus fruits produced by inoculations with known mixtures of fungi at different constant temperatures. Jour. Agr. Res. **39**: 163–198. 1929.
19. SMITH, R. E., *et al.* The brown rot of the lemon. California Agr. Expt. Sta. Bull. 190. 1907.
20. TUCKER, C. M. Taxonomy of the Genus Phytopthora de Bary. Missouri Agr. Expt. Sta. Res. Bull. 153. 1931.
21. VASUDEVA, R. S. Studies on the physiology of parasitism. XII. On the effect of one organism in reducing the parasitic activity of another. Ann. Bot. **44**: 557–564. 1930.

ACQUIRED IMMUNITY FROM CUCUMBER MOSAIC IN ZINNIA[1]

W. C. Price

(Accepted for publication May 13, 1935)

The problem of acquired immunity in plants has long held the attention of phytopathologists, but it is only recently that much consideration has been given the subject in connection with virus diseases. In the last few years, however, considerable evidence that plants may acquire immunity from virus diseases has been obtained. The work of McKinney (7), Thung (13), Salaman (12), Oortwijn Botjes (8), Kunkel (6), Ainsworth (1), and Caldwell (2) has shown that plants infected with one strain of a plant virus become immune from infection with other strains of the same virus. Moreover, evidence presented by Wingard (15) and Price (10) demonstrates that certain species of plants acquire immunity from tobacco ring spot, a disease from which they regularly recover. While considerably more work is necessary to determine the specificity of this acquired immunity, the results assembled up to the present time suggest that the immune reaction is similar to that which obtains in virus diseases of animals, and that it may prove applicable to plant viruses in general. It would be of fundamental importance from both the theoretical and practical standpoints if it could be shown that plants generally acquire immunity from virus diseases and an adequate technique could be found for demonstrating this immunity in a large number of such diseases. Aside from the purely theoretical aspects of the problem, a specific immune reaction would be of considerable value in differentiation and classification of plant viruses.

The reaction of *Zinnia elegans* Jacq. to strains of cucumber mosaic offers a means of testing for a specific cross-immunity reaction with cucumber-mosaic virus. Most strains of the virus cause a mottling disease in zinnia, but one has been found that produces necrotic primary lesions. The problem with which this paper is concerned is that of determining whether zinnia plants infected with mottling strains of cucumber mosaic become specifically immune from infection with the necrotic-type strain of the same virus. The value of using viruses that produce such distinctly different symptoms as mottling and necrosis, for studies on acquired immunity, becomes readily apparent when it is considered that the mottling symptoms of a virus first introduced into a host plant may very well obscure the symptoms of a second mottling virus introduced later, even though the symptoms of the latter be much more severe than those of the former. If, however, the second virus

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript.

is of the necrotic type, it produces lesions that can readily be detected even in leaves showing severe mottling symptoms.

In the experiments to be reported, it was found that zinnia plants mottled by certain strains of cucumber-mosaic virus are immune from infection with the necrotic-type strain of the same virus. On the other hand, the infection of zinnia with certain other viruses, such as tobacco mosaic, does not elicit immunity against this necrotic-type virus. Further evidence of the specificity of the immune reaction in zinnia was obtained by using a strain of tobacco mosaic that produces necrotic primary lesions in this host. Immunity from the necrotic-type tobacco-mosaic virus is conferred by a previous infection with ordinary tobacco- or aucuba-mosaic viruses, but it is not conferred by a previous infection with cucumber mosaic.

MATERIALS AND METHODS

Samples of virus used for inoculation were taken from diseased tobacco (*Nicotiana tabacum* L. var. Turkish) plants. The method of inoculation was the same in all cases. Leaves from diseased plants were macerated in a sterile mortar and a small volume of water was added. The solution thus obtained was taken up on a sterile cheese-cloth pad and was rubbed over the upper surface of several leaves of the plants to be inoculated. The leaves were rinsed with water shortly after inoculation.

The variety of *Zinnia elegans* used in the cross-immunity tests was Lilliput Golden Gem, seed of which were obtained from Peter Henderson and Company, New York. Plants were grown in a greenhouse in which the temperature did not fall below 65° F.

DESCRIPTION OF VIRUSES AND SYMPTOMS PRODUCED IN ZINNIA

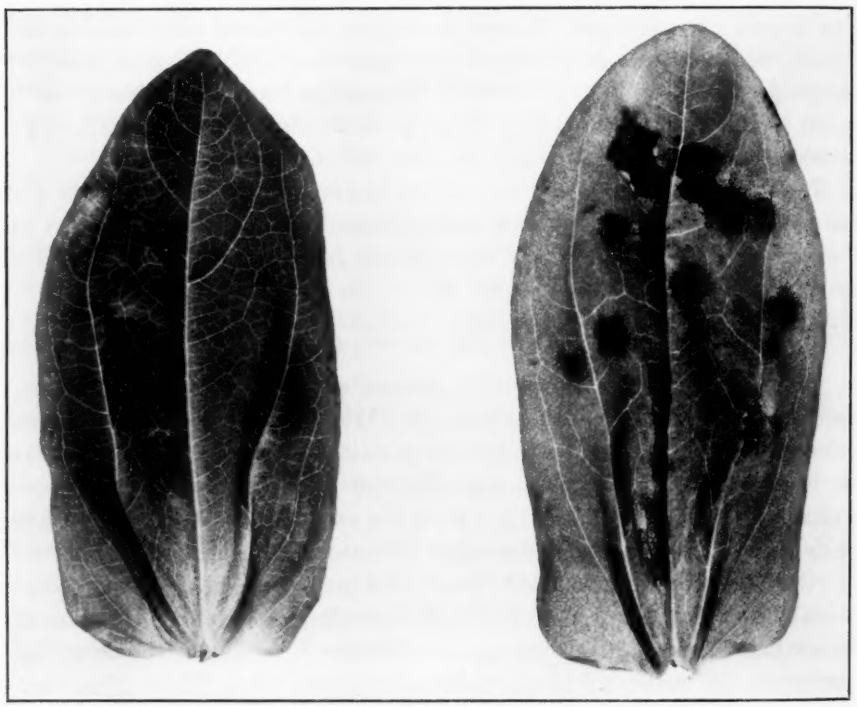
The various strains of cucumber mosaic used in the present study have been described in a previous publication (11). Ordinary cucumber mosaic, referred to here as Porter's cucumber mosaic, was obtained originally from R. H. Porter (9). The other strains are believed to be derivatives of Porter's cucumber mosaic (11). With the exception of strain 6, which will be described later, the cucumber-mosaic viruses tested produce the clearing-of-veins symptom and mottled leaves in *Zinnia elegans*. The clearing-of-veins symptom appears in from 6 to 8 days after inoculation, while mottled leaves make their appearance on the 10th to 12th day. The mottling is similar to that produced in cucumber and certain other hosts. The various strains differ principally in the intensity of the mottling symptoms. Strain 2 produces a brilliant yellow and green mottling, whereas strain 1, strain 9,[2]

[2] This strain was referred to in a previous paper (11) as strain Y. Because of the possibility of confusion with the Y virus of potato, it will hereafter be designated as strain 9.

and Porter's cucumber mosaic produce a mottling of light and dark green. Figure 1 shows the symptoms produced by Porter's cucumber-mosaic virus. Plants infected with any of the mottling strains are considerably stunted but only slightly distorted.

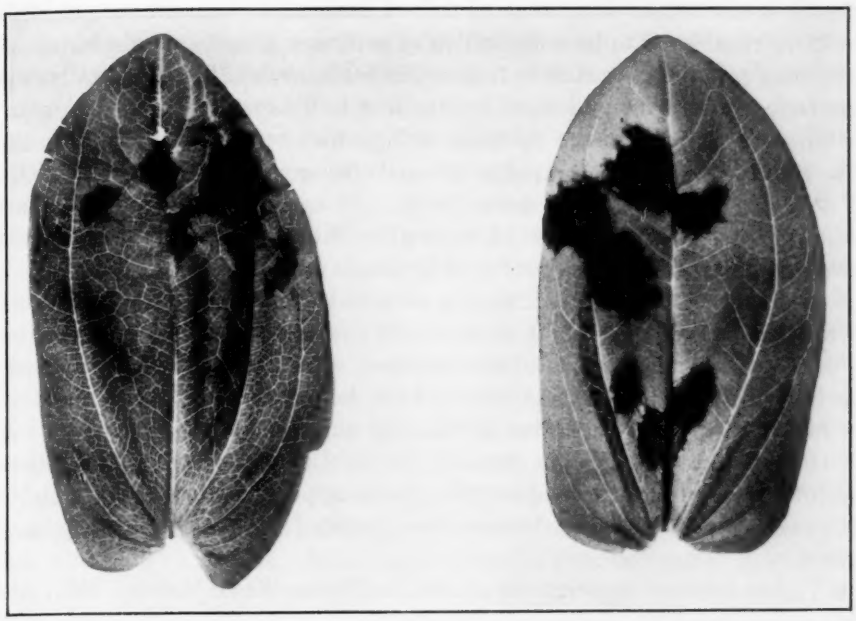
When transferred to zinnia, cucumber mosaic strain 6 induces the formation of bright yellow primary lesions, which appear usually 3 or 4 days after inoculation, soon become necrotic and rapidly increase in size (Figs. 1 and 2). The necrotic lesions are dark brown. In some cases they may involve the entire leaf in which they occur. The necrosis frequently spreads down the petiole into the stem causing elongated, dark brown stem lesions. Occasionally the virus spreads to the tip of the plant, producing necrotic spots similar to those that occur in inoculated leaves. Plants that develop stem lesions usually are killed.

Ordinary tobacco-mosaic virus is readily transmitted to zinnia. Infected plants may develop small, chlorotic, primary lesions in 3 to 5 days after



Photographed by J. A. Carlile

FIG. 1. Lesions produced by inoculation of zinnia leaves with virus of cucumber mosaic strain 6. The leaf on the left was previously mottled by Porter's cucumber mosaic and did not develop lesions. The leaf on the right was a previously healthy control and shows numerous lesions. Porter's cucumber mosaic protects zinnia plants from infection with cucumber mosaic strain 6.



Photographed by J. A. Carlile

FIG. 2. Lesions produced by inoculation of zinnia leaves with virus of cucumber mosaic strain 6. The leaf on the left was previously infected with severe-etch virus. That on the right was a healthy control. Virus of cucumber mosaic strain 6 has produced necrotic primary lesions in both leaves. Severe etch does not protect zinnia plants from infection with cucumber mosaic strain 6.

inoculation, but most plants do not show evidence of primary infection. The clearing-of-veins symptom has been observed in a few plants on the 7th day following inoculation. The most characteristic symptom, however, is a light and dark green mottling of leaves. This is shown rarely by young tip leaves, most conspicuously by leaves just below the tip, and only faintly by older leaves. Plants in which mottling fails to develop show characteristic chlorotic spots similar to those described by Grant (3). Infected plants are usually severely stunted by the disease.

The symptoms of aucuba mosaic in zinnia are in many respects similar to those produced by tobacco mosaic. The mottling pattern is, however, much more conspicuous than is the case with the latter disease. It is composed of areas of various shades of yellow intermingled on a dark green background. Affected leaves frequently develop pale to brilliant yellow zonate ring patterns.

The necrotic-type tobacco-mosaic virus used in the present study was isolated from a yellow-mosaic virus supplied by J. H. Jensen (4) and designated by him as strain 302. In this paper it will be referred to as tobacco

mosaic strain 302A. It is believed to be a derivative of strain 302, which is, in turn, considered to be a derivative of ordinary tobacco-mosaic virus. It produces conspicuous primary lesions in *Zinnia elegans* but does not become systemic. The primary lesions appear in 4 to 6 days after inoculation and enlarge slowly. They are chlorotic at first and become necrotic with age. The necrosis may develop rapidly, the spots being completely necrotic within 6 days, or it may develop more slowly. In many cases the lesions have remained chlorotic for 12 to 14 days after inoculation. Figures 3 and 4 show characteristic lesions produced by strain 302A in zinnia.

The tobacco-ring-spot virus was obtained from S. A. Wingard (15). When transferred to zinnia it produces the clearing-of-veins symptom within 5 or 6 days after infection. This symptom is accompanied by a downward curling or rolling of affected leaves; such leaves tend to blanch and turn yellow at their bases. Leaves developing subsequently may show a mild mottling of green and light green. Ten to 12 days after infection these symptoms are no longer evident; the plants appear healthy or show only a faint mottling. Virus may be recovered readily from the healthy-appearing leaves.

Yellow tobacco-ring-spot virus, obtained from W. D. Valleau (14), produces symptoms in zinnia quite similar to those of Wingard's ring spot. The symptoms are, however, somewhat more severe. Some plants show bright yellow, irregular areas in leaves just below the tip of the plant, while others recover and appear to be healthy after the first onset of the disease.

Severe etch has been described by E. M. Johnson (5). When transferred to zinnia it produces a marked clearing-of-veins symptom (Fig. 2). This symptom persists more or less throughout the course of the disease. In addition, affected leaves are twisted and distorted. They do not develop the typical mottling symptoms characteristic of the mosaic viruses. Infected plants are, however, severely stunted.

THE IMMUNE REACTION IN ZINNIA

Neither cucumber-mosaic strain 6 nor tobacco-mosaic strain 302A produces large numbers of necrotic lesions in leaves of *Zinnia elegans* inoculated by the rubbing method. The maximum number so far produced in a single leaf is approximately 50, while usually only 20 or less are produced. The numbers of lesions vary greatly within a given set of inoculated zinnia plants, and the variation is even greater in different sets of plants. The reason for the variability is obscure, but it is believed that the numbers of lesions may be influenced by the condition of the test plants and by the source of the inoculum. Because of the variation in numbers of lesions in a given set of plants and because of the failure to obtain large numbers of primary infections, it was considered essential to use a large number of control plants

and to repeat the experiments a number of times before drawing definite conclusions. Accordingly, each of the experiments reported here was repeated several times. Except for slight quantitative differences, there was a close agreement between the repetitions of the same experiment.

Leaves Mottled by Cucumber-mosaic Virus are Immune from Infection with Virus of Strain 6. An experiment was performed for the purpose of determining whether or not zinnia leaves that are mottled by any one of 4 different strains of cucumber-mosaic virus are immune from infection with virus of strain 6. Ten young zinnia plants were inoculated with virus of Porter's cucumber mosaic, 10 with virus of strain 1, 10 with virus of strain 2, and 10 with virus of strain 9. Eighteen days after inoculation several leaves on each of the inoculated plants were mottled with green and yellow blotches, while the noninoculated control plants remained healthy. Six of the mottled leaves on each of the diseased plants and 6 comparable leaves on each of the control plants were thoroughly inoculated with virus of cucumber mosaic strain 6. Lesions did not appear in any of the mottled leaves during a period of more than 2 weeks that they were kept under observation. The control plants, with 2 exceptions, developed a number of necrotic primary lesions. The necrotic spots were counted, and the results obtained are given in table 1. The consistency with which lesions developed in healthy leaves, and the complete freedom of mottled leaves from such lesions give conclusive evidence of the immunity of mottled leaves,

TABLE 1.—*Numbers of lesions produced by inoculation of mottled leaves and of healthy leaves of Zinnia elegans with virus of cucumber mosaic strain 6*

Previous condition of test plants	Plant number										Total
	1	2	3	4	5	6	7	8	9	10	
Infected with Porter's cucumber mosaic	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	161	78	105	57	156	38	75	164	108	58	1000
Infected with strain 1	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	13	9	23	10	28	18	8	6	9	12	136
Infected with strain 2	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	10	16	6	21	0	11	8	4	18	3	97
Infected with strain 9	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	9	11	3	10	12	6	0	16	7	8	82

in spite of the fact that only a small number of lesions was produced in some of the controls. The immunity against strain 6 afforded by a previous infection with Porter's cucumber mosaic is illustrated in figure 1. It is concluded that zinnia plants that are mottled by Porter's cucumber mosaic, cucumber mosaic strain 1, strain 2, or strain 9 are immune from infection with virus of cucumber mosaic strain 6.

Invasion of Protecting Viruses Necessary for Complete Immunization of Plants against Strain 6. In the experiment just reported, virus of the mottling strains of cucumber mosaic was allowed to become thoroughly systemic before testing for immunity. It appeared desirable to determine whether or not plants that had been infected for a shorter period of time and in which the virus had not become thoroughly systemic would likewise be immune from infection with strain 6. Ten young zinnia plants were inoculated with Porter's cucumber-mosaic virus, 10 were inoculated with strain 1, 10 with strain 2, and 10 with strain 9. An equal number of plants were kept uninoculated and served as controls. On the 11th day after inoculation the plants showed the clearing-of-veins symptom. Four leaves near the tip of each of the diseased plants were then thoroughly inoculated with virus of strain 6. An equal number of control leaves were inoculated with the same virus samples. Lesions appeared in the test plants in leaves that showed the clearing-of-veins symptom, and also in healthy appearing leaves. Table 2 shows the total numbers of lesions produced in the test and

TABLE 2.—*Numbers of lesions produced by inoculation of zinnia plants with virus of cucumber mosaic strain 6. The test plants had been previously infected with ordinary cucumber mosaic and were just showing the clearing-of-veins symptom. The controls were previously healthy plants*

Virus tested	Number of lesions	
	Test plants	Controls
Porter's cucumber mosaic	24	186
Strain 1	12	170
Strain 2	21	105
Strain 9	10	42

control plants. An examination of the table reveals that lesions were far more numerous in the previously healthy plants than in previously infected plants. Some immunity was shown by the test plants, but it was not complete. The results indicate that acquired immunity from strain 6 is confined to tissues actually invaded by the protecting virus.

As a further check to the experiment, the inoculated leaves showing lesions were removed and the diseased plants allowed to become thoroughly

mottled. On the 28th day after the first inoculation, both diseased and control plants were inoculated with virus of strain 6. None of the plants infected with either Porter's cucumber mosaic, strain 1, strain 2, or strain 9 developed lesions. A total of 136 lesions appeared in the controls.

Failure to Immunize Inoculated Leaves. It has been shown by Kunkel (6) that leaves of *Nicotiana sylvestris* Speg. and Comes inoculated with tobacco-mosaic virus become immune from aucuba-mosaic virus. An experiment was, therefore, undertaken in order to determine whether zinnia leaves inoculated with Porter's cucumber-mosaic virus likewise become immune from cucumber mosaic strain 6. Six leaves on each of 30 healthy zinnia plants were thoroughly rubbed with juice containing Porter's cucumber-mosaic virus. At the same time, 6 leaves on each of 30 similar plants were rubbed with water. The latter set of 30 plants was used as controls. After 6 days the rubbed leaves of each of the 60 plants were inoculated with cucumber mosaic strain 6. A total of 1118 necrotic lesions developed in the leaves that were previously inoculated with Porter's cucumber mosaic. A total of 1345 necrotic lesions appeared in the controls. The results show that zinnia leaves previously inoculated with ordinary cucumber-mosaic virus were not fully protected from infection with strain 6. The results of a previous experiment have shown that the invasion of tissues by the protecting viruses is necessary for their complete immunization against strain 6. It is, therefore, believed that the failure to obtain immunization of leaves inoculated with Porter's cucumber mosaic resulted from inability to produce the numerous primary infections that are necessary for complete invasion of the tissues of inoculated leaves.

SPECIFICITY OF THE IMMUNE REACTION

The failure of zinnia plants mottled by certain strains of cucumber-mosaic virus to develop lesions when inoculated with virus of cucumber mosaic strain 6 demonstrates that such plants are immune from the latter virus. It seemed desirable, however, to determine whether this immunity is or is not specific. Two types of experiment were, therefore, conducted. Firstly, tests were made with a number of viruses believed to be unrelated to cucumber mosaic in order to determine whether or not they protect zinnia plants from infection with cucumber mosaic strain 6. Secondly, reciprocal tests were made to determine whether or not zinnia plants mottled by cucumber mosaic, or certain other viruses, are completely protected against infection with the necrotic-type tobacco mosaic, strain 302A.

Viruses Believed to be Unrelated to Cucumber Mosaic do not Confer Immunity from Strain 6. Sets of 10 young zinnia plants were inoculated with the following viruses: tobacco mosaic, aucuba mosaic, severe etch, tobacco ring spot, and yellow ring spot. An equal number of similar plants

were not inoculated and served as controls. All the inoculated plants became diseased and developed symptoms characteristic for the virus with which they were infected. Eighteen days after inoculation, 6 infected leaves on each of the diseased plants and 6 leaves on each of the healthy controls were rubbed with juice containing virus of cucumber mosaic strain 6. Necrotic lesions developed in the diseased plants, as well as in the controls. The numbers of lesions are shown in table 3. From this table it will be seen

TABLE 3.—*Numbers of lesions produced by inoculation of infected leaves and of healthy leaves with virus of cucumber mosaic strain 6*

Previous condition of test plants	Plant number										Total
	1	2	3	4	5	6	7	8	9	10	
Infected with tobacco mosaic	7	0	0	9	1	2	1	1	0	4	25
Healthy controls	1	11	18	7	6	1	17	4	9	24	98
Infected with aucuba mosaic	12	22	9	12	17	38	12	1	5	7	135
Healthy controls	22	4	6	1	20	1	4	19	10	14	101
Infected with severe etch	6	0	3	1	5	6	1	9	0	4	35
Healthy controls	1	4	4	1	0	3	5	7	6	1	32
Infected with tobacco ring spot	3	19	5	3	13	2	5	15	9	1	75
Healthy controls	10	17	24	6	13	12	41	8	12	18	161
Infected with yellow ring spot	10	13	13	4	20	8	15	10	8	9	110
Healthy controls	44	27	10	16	68	9	21	4	12	17	228

that the plants having tobacco mosaic, tobacco ring spot, and yellow ring spot developed fewer lesions than the controls, but that those infected with aucuba mosaic showed more lesions than the corresponding controls. In the case of most of the viruses tested, the lesions appeared equally early in both the diseased and healthy plants. However, in the case of severe etch, the lesions in the diseased plants required 8 days to become completely necrotic as compared with 5 days for the lesions in the controls. Infection of zinnia plants with tobacco mosaic, aucuba mosaic, tobacco ring spot, yellow ring spot, or severe etch did not confer immunity against cucumber mosaic strain 6. The failure of severe-etch virus to immunize zinnia plants against cucumber mosaic strain 6 is illustrated in figure 2. The results indicate that

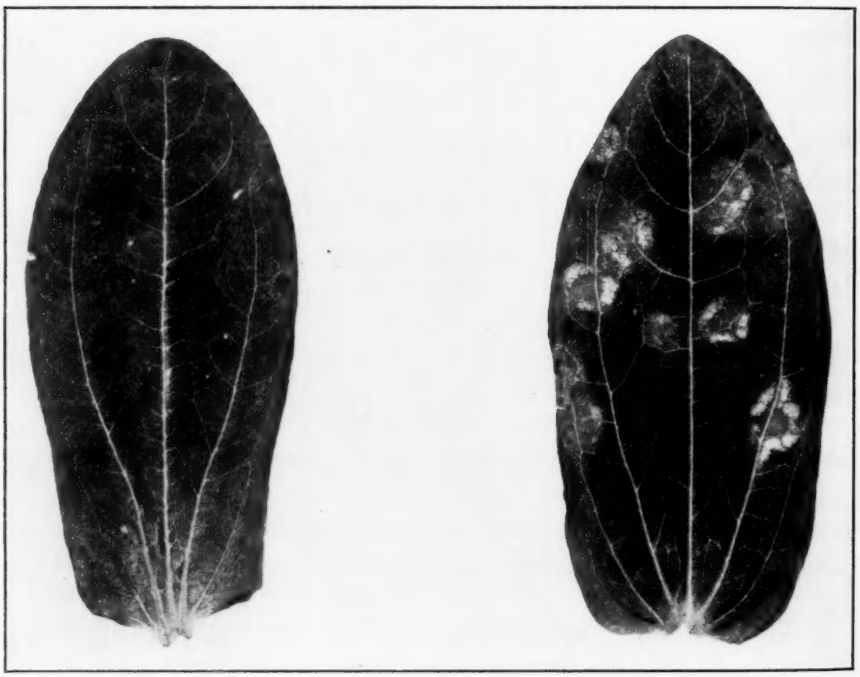
the immune reaction against virus of strain 6 is confined to plants infected with strains of cucumber-mosaic virus.

Reciprocal Cross-immunity Tests in Zinnia. In conducting the reciprocal cross-immunity tests, use was made of tobacco mosaic strain 302A, a necrotic-type virus in zinnia. Young zinnia plants were divided into sets of 10 each and inoculated with the following viruses: tobacco mosaic, aucuba

TABLE 4.—*Numbers of lesions produced by inoculation of infected leaves and of healthy leaves with virus of tobacco mosaic strain 302A*

Previous condition of test plants	Plant number										Total
	1	2	3	4	5	6	7	8	9	10	
Infected with tobacco mosaic	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	15	10	18	8	18	3	29	26	15	9	151
Infected with aucuba mosaic	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	16	1	3	21	10	32	44	3	14	–	144
Infected with cucumber mosaic	0	13	1	1	8	4	5	16	4	2	54
Healthy controls	0	4	2	10	10	6	4	7	9	8	60
Infected with strain 1	11	10	0	19	24	11	3	9	30	25	142
Healthy controls	13	11	36	45	19	13	13	50	19	15	234
Infected with strain 2	2	4	12	9	39	13	5	0	8	23	115
Healthy controls	18	45	3	8	21	8	13	32	8	13	169
Infected with strain 9	20	2	7	15	5	11	5	11	10	0	86
Healthy controls	17	16	14	18	21	13	33	3	32	6	173
Infected with tobacco ring spot	14	41	29	17	0	18	9	16	16	13	173
Healthy controls	51	38	15	45	31	3	13	13	3	40	252
Infected with yellow ring spot	21	38	12	34	34	11	63	34	6	20	273
Healthy controls	27	30	13	21	28	10	35	34	9	22	229
Infected with severe etch	13	10	10	7	28	4	6	15	30	11	134
Healthy controls	20	11	33	7	10	6	22	14	13	38	174

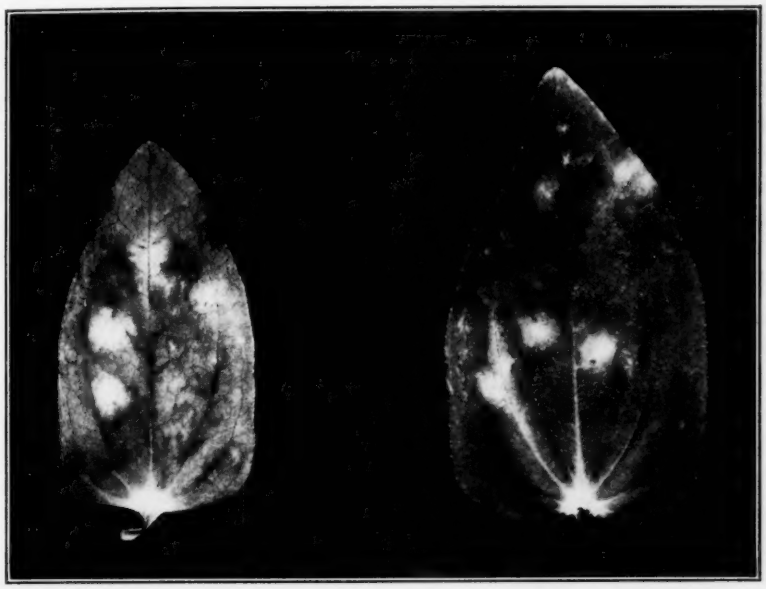
mosaic, Porter's cucumber mosaic, cucumber mosaic strain 1, cucumber mosaic strain 2, cucumber mosaic strain 9, tobacco ring spot, yellow ring spot, and severe etch. All the inoculated plants became infected and developed the usual symptoms. An equal number of similar plants left non-inoculated and used for controls remained healthy. Sixteen days after inoculation, 4 infected leaves on each of the diseased plants and 4 leaves on each of the controls were inoculated with virus of tobacco mosaic strain 302A. No lesions developed in any of the plants infected with either tobacco mosaic or aucuba mosaic, but the lesions were numerous in the controls and in the plants infected with the other viruses, as shown in table 4. The results show, firstly, that zinnia plants that are infected with tobacco- or aucuba-mosaic virus become immune from infection with virus of tobacco mosaic strain 302A, and, secondly, that zinnia plants infected with ordinary cucumber-mosaic virus or with any one of 3 other strains of this virus, or with tobacco-ring-spot, yellow-ring-spot, or severe-etch virus do not acquire immunity from virus of tobacco mosaic strain 302A. Figure 3 illustrates



Photographed by J. A. Carlile

FIG. 3. Lesions produced by inoculation of zinnia leaves with virus of tobacco mosaic strain 302A. The leaf on the left was previously infected with aucuba mosaic and did not develop lesions. The leaf on the right was previously healthy and developed a number of lesions. Aucuba mosaic protects zinnia plants from infection with tobacco mosaic strain 302A.

the immunity against virus of tobacco mosaic strain 302A induced by a previous infection with aucuba-mosaic virus. The failure of cucumber-mosaic virus to elicit immunity against virus of tobacco mosaic strain 302A is illustrated in figure 4. The results bring additional evidence that the immune reaction in zinnia is specific.



Photographed by J. A. Carlile

FIG. 4. Lesions produced by inoculation of zinnia leaves with virus of tobacco mosaic strain 302A. The leaf on the left was previously infected with Porter's cucumber mosaic. The leaf on the right was previously healthy. Both leaves have developed several lesions. Porter's cucumber mosaic does not protect zinnia plants from infection with tobacco mosaic strain 302A.

DISCUSSION

The results presented in this paper are based, for the most part, on infections resulting in small numbers of lesions. They are, nevertheless, considered to be significant. The tests show that zinnia plants infected with mottling strains of cucumber-mosaic virus become immune from infection with a necrotic-type strain of this virus. They show, moreover, that this immunity is specific and does not extend to any of the unrelated groups of plant viruses. The immune reaction in zinnia allows a classification of the 5 cucumber-mosaic viruses into a distinct group and the 3 tobacco-mosaic viruses into a second distinct group. Further evidence is needed, however, for determining the relationships of severe etch, tobacco ring spot, and

yellow tobacco ring spot. None of these viruses conferred immunity against either tobacco mosaic or cucumber mosaic.

The mechanism by which zinnia plants become immune from cucumber mosaic strain 6 has not been revealed. It is known, however, that this immunity is associated with the presence of the protecting virus in tissue areas that acquire immunity. Uninvaded leaves on plants infected with mottling-type strains of cucumber-mosaic virus are susceptible to infection with the virus of strain 6. For this reason, it is necessary to determine whether or not the virus under test has become thoroughly systemic before definite conclusions can be reached. In the present work in which zinnia plants were inoculated with different viruses, the infected plants were allowed to become thoroughly invaded before being tested for immunity against either cucumber mosaic strain 6 or tobacco mosaic strain 302A.

Zinnia elegans is not an ideal plant for use in a study of acquired immunity from cucumber- or tobacco-mosaic viruses. It does, however, offer certain advantages that are not possessed by any other host plant, so far as known. The greatest advantage lies in the fact that within both the cucumber- and tobacco-mosaic groups strains have been found that produce primary necrotic lesions in zinnia, whereas the other strains tested produce mottling diseases. This difference in reaction is admirably adapted to cross-immunity studies and allows a severe test to be made of the specificity of the reaction. Zinnia also possesses the advantage of being susceptible to a number of different virus diseases and thereby allows a further extension of the work on differentiation and classification. On the other hand, the susceptibility of zinnia to the viruses employed is much lower than might be desired. This difficulty has been overcome by using a large number of plants and by repeating the tests a number of times.

SUMMARY

A description is given of the symptoms produced in *Zinnia elegans* by a necrotic-type cucumber-mosaic virus and by a necrotic-type tobacco-mosaic virus.

Zinnia plants that are mottled by any one of 4 different strains of cucumber-mosaic virus become immune from infection with a necrotic-type strain of this virus, but are susceptible to a necrotic-type tobacco-mosaic virus. Similarly, plants that are mottled by tobacco- or aucuba-mosaic virus become immune from a necrotic-type tobacco-mosaic virus, but do not develop immunity against a necrotic-type cucumber-mosaic virus. On the other hand, plants infected with tobacco-ring-spot, yellow-ring-spot, or severe-etch virus do not acquire immunity either from the necrotic-type cucumber-mosaic virus or from the necrotic-type tobacco-mosaic virus.

Evidence is thus obtained of the specificity of the immune reaction in zinnia for both cucumber- and tobacco-mosaic viruses.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED

1. AINSWORTH, G. C. A comparison of certain English and Canadian potato viruses found infecting tomatoes. Ann. Appl. Biol. **21**: 581–587. 1934.
2. CALDWELL, JOHN. On the interactions of two strains of a plant virus; experiments on induced immunity in plants. Proc. Roy. Soc. Ser. B, **117**: 120–139. 1935.
3. GRANT, THEODORE J. The host range and behavior of the ordinary tobacco-mosaic virus. Phytopath. **24**: 311–336. 1934.
4. JENSEN, JAMES H. Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. Phytopath. **23**: 964–974. 1933.
5. JOHNSON, E. M. Virus diseases of tobacco in Kentucky. Kentucky Agr. Exp. Sta. Bul. (Res. Bul.) **306**. 1930.
6. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. **24**: 437–466. 1934.
7. MCKINNEY, H. H. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. Jour. Agr. Res. **39**: 557–578. 1929.
8. OORTWIJN BOTJES, J. G. Verzwakking van het virus der topnecrose, en verworven immuniteit van aardappelrassen ten opzichte van dit virus. Tidschr. Plantenz. **39**: 249–262. 1933.
9. PORTER, R. H. The reaction of cucumbers to types of mosaic. Iowa State Coll. Jour. Sci. **6**: 95–119. 1931.
10. PRICE, W. C. Acquired immunity to ring-spot in Nicotiana. Contrib. Boyce Thompson Inst. **4**: 359–403. 1932.
11. ————. Isolation and study of some yellow strains of cucumber mosaic. Phytopath. **24**: 743–761. 1934.
12. SALAMAN, REDCLIFFE N. Protective inoculation against a plant virus. Nature **131**: 468. 1933.
13. THUNG, T. H. Smetstof en plantencel bij enkele virusziekten van de tabaksplant. Handeling. Nederl.-Ind. Natuurwetenschap. Cong. 6de **1931**: 450–463. 1931.
14. VALLEAU, W. D. Seed transmission and sterility studies of two strains of tobacco ringspot. Kentucky Agr. Exp. Sta. Bul. (Res. Bul.) **327**: 43–80. 1932.
15. WINGARD, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. **37**: 127–153. 1928.

MORPHOLOGIC STUDIES OF SEPTORIA LYCOPERSICI[1]

HUBERT A. HARRIS[2]

(Accepted for publication December 1, 1934)

This study was not occasioned by belief that the existent morphologic investigations of *Septoria lycopersici* Speg., the tomato leaf-spot fungus, were inadequate. It was, instead, purported to confirm, if possible, certain developmental features of the fungus and perhaps to increase the present knowledge of the organism by additional facts that might present themselves during the course of investigation.

These morphologic observations include: (1) character of the vegetative mycelium; (2) developmental stages of pycnidial and pycnospore formation; (3) comparative studies of pycnospore germination in hanging-drop culture and on the host plant; and (4) relationship between the fungus and the host tissue, including infection penetration.

MATERIALS AND METHODS

The fungus used in this study was isolated from garden-grown tomato plants in Urbana, Illinois. The plants were moderately infected with Septoria leaf spot, and on the date of collection, October 5, 1931, the leaves bore numerous spots containing mature pycnidia exuding masses of pycnospores.

A single spore isolation of the fungus was made according to the method outlined by Davis (2), and the resultant cultural growth was that used for inoculation purposes.

Separate leaflets of several potted tomato plants were inoculated with a single loopful of a rather concentrated pycnospore suspension in sterile distilled water. The method employed by Levin (6) of placing a small, thin piece of cotton over the point of inoculation also was adopted. The cotton not only served as an exact means for locating the inoculation point, but also prevented the removal of pycnospores due to the condensation of moisture on the leaves, since the inoculated plants were placed in humidity cases for infection development.

Histological material was secured by taking 1 sq. cm. of leaf tissue from the inoculated points for 12 successive days. The tissue was killed

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Botany in the graduate school of the University of Illinois, 1932.

[2] The writer wishes to express grateful acknowledgment for the kindly suggestions and indispensable advice given by the late Professor F. L. Stevens under whose supervision this work was done.

and fixed in strong chromo-acetic-acid solution, and the chloroform method of infiltration was followed. Paraffin sections of the imbedded leaf tissue were cut 5 to 8 μ in thickness.

To secure differential staining of fungus and host tissue, Pianeze III B (8), and Dickson's stain (3) were tried. Effective differentiation could not be secured with the former stain, inasmuch as the parenchymatous

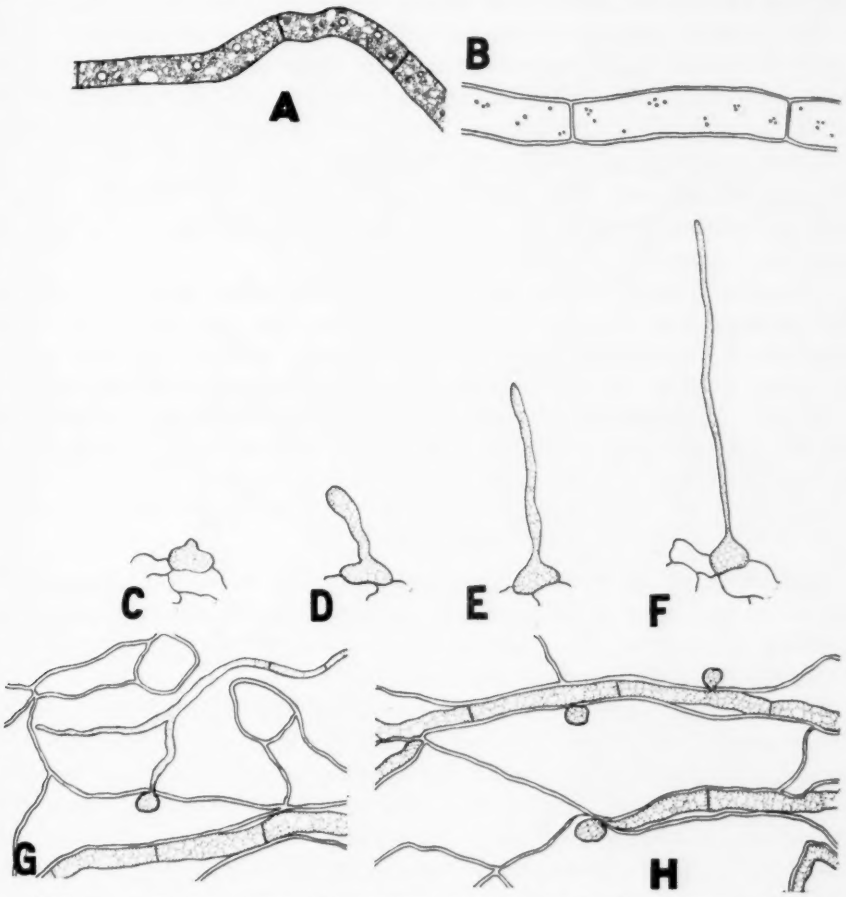


FIG. 1. A–B. Two types of mycelium occurring in the host tissue. ×1250. A. Hyaline, thin-wall type. B. Brown, thick-wall type. C–F. Progressive stages of pycnospore formation. ×1250. C. Initial stage showing the small, papillate protrusion from a cell of the undifferentiated sporogenous tissue. D. and E. Successive stages of the elongative basipetal development of pycnospores. F. Nearly mature pycnospore prior to its constrictive separation from the sporogenous cell. G. Cross section of stomatal penetration by germinating pycnospore on the upper leaf surface after 60 hours. ×1000. H. Intercellular mycelium of the fungus showing penetration of the host cells by blunt, globular haustoria. ×1000.

tissue of the tomato leaf did not retain the malachite green, but stained red, like the fungus. Excellent differentiation was secured, however, with Dickson's combination stain.

MYCELIUM

The mycelium of *Septoria lycopersici* is of 2 types: (1) the hyaline, thin-wall type (Fig. 1, A), and (2) the brown, thick-wall type (Fig. 1, B).

The hyaline mycelium is characteristic of the young and actively growing hyphae, and predominates during the early progress of the fungus through the leaf tissue succeeding initial infection. The sporogenous tissue within the pycnidium also consists of this type of mycelium. The hyphal walls are quite thin, hyaline, and infrequently septate, though occasionally close septation occurs. Protoplasmic contents are densely granular, vacuolated, and contain numerous small oil globules. The diameter of the hyaline mycelium ranges from 1.2 to 5.8 μ.

The brown mycelium is characteristic of the older mycelium, and is also the type that enters into the formation of the pycnidial wall. This mycelium is infrequently branched, and usually exhibits close septation. Its brown hyphal walls are about twice as thick as those of the hyaline mycelium. Protoplasmic contents are almost imperceptible, though occasionally large vacuoles and a few scattered oil globules occur. A diameter of 2.2 to 5.8 μ has been noted for this type of mycelium.

PYCNIDIAL FORMATION

Levin studied the mode of pycnidial formation of *Septoria lycopersici*, and found it to be symphyogenous A study of the fungus in the present investigation confirms the report of Levin, and in no instance has evidence of meristogenous development been observed.

In the initial stage of pycnidial formation, several hyphae aggregate and intersect in the leaf tissue (Fig. 2, A). Here the hyphal tips are just beginning to bend and intertwine preparatory to the formation of a pycnidial primordium (Fig. 2, B). In this early primordial stage, the intertwining hyphae have formed a conglobate mass of hyphal tissue that already has begun to assume the appearance of pseudoparenchyma. Figure 2, C, shows a somewhat later primordial stage. Continued division and aggregation of hyphal cells have occurred, and, in this instance, in close proximity to the vascular tissue of the leaf. The next stage to be observed in pycnidial formation is the development of a pycnidial chamber (Fig. 2, D). This central cavity is just beginning to form, and all observational evidence indicates that it results from a schizogenous process in which the central hyphae or hyphal cells are pulled apart because the peripheral primordial growth is greater than the interior growth. Figure 2, E, shows

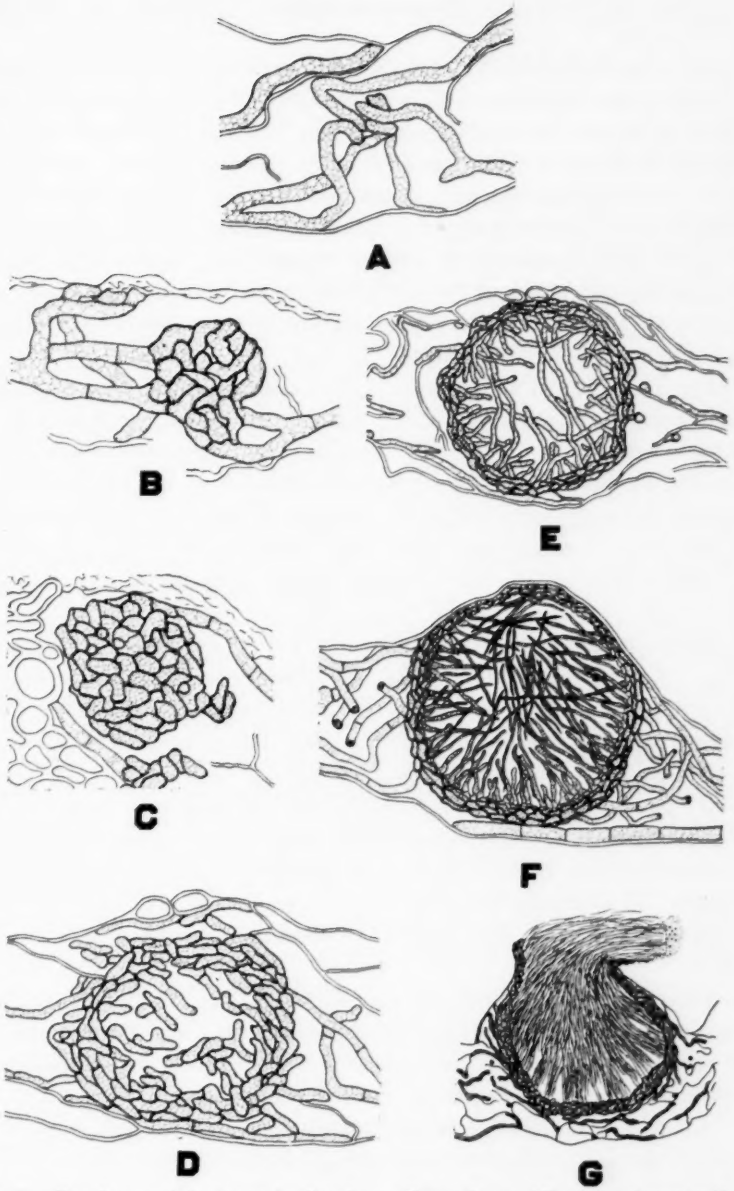


FIG. 2. Developmental stages in the formation of pycnidia. A. Aggregation and intersection of hyphae preparatory to the symphyogenous formation of the pycnidial primordium. ×1000. B. Early stage in the development of the pycnidial primordium. ×1000. C. Later stage in the formation of a pycnidial primordium showing the continued development of pseudoparenchymatous tissue. ×550. D. Young pycnidium showing the appearance of the pycnidial chamber during its early formation. ×900. E. Later stage of pycnidial development showing the pycnidial chamber prior to ostiolar formation, and with several hyphae still extending across it. ×500. F. Pycnidium in the initial stage of pycnospore formation previous to ostiolar formation. ×400. G. Fully mature pycnidium, in which extrusion of pycnospores has occurred through the wide ostiole. ×225.

this central pycnidial cavity well-developed, though mycelial strands still extend across the chamber. In this stage a definite pycnidial wall is seen to exist and to consist of compacted peridial cells. Figure 2, F, shows a pycnidium in which pycnospore formation has just begun, and numerous, separate pycnospores already occur in the pycnidial chamber. This pycnidium also shows a stage of development that will be treated further in the subsequent discussion of ostiolar formation. Figure 2, G, represents the most advanced stage of pycnidial development, and this fully mature pycnidium is characterized by the exudation of great masses of pycnospores through the conspicuously wide ostiole.

Archer (1) studied the morphological characters of several Sphaeropsidales in culture, including *Septoria lycopersici,* and ascertained some additional facts relative to pycnidial formation that were unreported by Levin. He found that, just preceding pycnospore formation, the hyphae extending across and through the pycnidial chamber undergo dissolution to form a dense, vacuolated, gelatinous substance. This gelatinous material, which fills the pycnidial cavity, is displaced when pycnospores are formed and forced into it.

In the present investigation a pycnidial stage in which a general gelatinization of hyphae occurred was not observed. Close examination of several pycnidial stages, prior to pycnospore formation, did show, however, fragmental hyphae evidencing dissolution. These hyphal fragments were quite swollen and irregular, and portions of them were almost imperceptible, since they were in a partial state of disintegration. Another fact tending to substantiate hyphal gelatinization was the release of a hyaline, granular substance at the same time pycnospores were exuded through the ostiole. This substance (Fig. 2, G) was forced out together with the pycnospore mass, and apparently represents the disintegrated hyphal material.

Diedicke (4), supported by the investigations of Klebahn (5) and Potebnia (7), reported that some species of Septoria have more or less broad, open-top fructifications from which a peridium arises and partially surrounds the inner sporogenous tissue. Potebnia has designated such a structure, a pseudopycnidium, and Diedicke excluded all forms lacking this pseudopycnidium as true Septorias. According to Diedicke, *Septoria lycopersici* shows neither a pseudopycnidial stage nor a preformed ostiole at any time during its development, and, therefore, could not be classified as a Septoria. Archer is opposed to this pseudopycnidial distinction by Potebnia and Diedicke, and states: "The writer considers there has not been a single fact presented to prove that the pycnidial wall does form in this way or that the fruiting body of Septoria is any more 'pseudopycnidial' than those of Sphaeropsis of Phoma species."

Pycnidial formation of *Septoria lycopersici* is not definitely positioned in the host tissue, as is reported for some species of Septoria. Weber (9) found that *Septoria nodorum* Berk., and *Septoria tritici* Desm. developed pycnidia only in the substomatal chambers. In a few instances this has been observed also to occur with *Septoria lycopersici.* However, the pycnidia of this fungus also form in the spongy tissue of the leaf and, frequently, directly beneath stomata (Fig. 2, D and E).

The pycnidia do not form simultaneously, but various stages of development occur successively. Thus, pycnidia were first observed forming 6 days after inoculation, but they were also still continuing to form in material examined 12 days after inoculation. On this latter date, mature pycnidia exuding spores were also present in the same material.

OSTIOLAR FORMATION

Levin (6) stated that two forces operated in forming the ostiole of *Septoria lycopersici:* (1) that brought about by the tension between the epidermis and enlargement of the pycnidium, and (2) that exerted by the pycnospore mass on the pycnidial wall at the point of greatest tension.

Archer's examinations (1) of the ostiolar formation of the fungus led him to believe that another factor also is involved in the process, namely, a dissolving factor. His evidence consists of the presence of separated, brown, thick-wall primordial cells lying free within or else lining the pycnidial cavity. These cells exhibited every appearance of having been partially dissolved. This dissolving action, combined with the internal force exerted on the pycnidial wall, would account for the wide and irregular ostiole characteristic of *Septoria lycopersici.*

The conclusions of Levin (6) concerning ostiolar formation have apparently been confirmed in the present study, but no evidence has been observed to substantiate the lysigenous action reported by Archer. It is not to be implied, however, that such lysigenous action does not occur, but rather that it was not observed in the material from which this present study was made.

It is obvious that the pycnidium shown in figure 2, F, is exerting a tension on the epidermal layer of the leaf, since this is greatly extended by enlargement of the pycnidial wall. This force, together with that to be exerted by the great mass of pycnospores beneath the point of ostiolar formation, would, however, seem sufficient to rupture the pycnidial wall, even if no lysigenous action were involved. That ostiolar formation involves a tensional relationship, was substantiated by cultural growth of the fungus on agar by both Levin (6) and Archer (1). Often, the weakest point of the pycnidial wall was submerged in the agar, and pycnospore

masses were frequently observed to extrude into the agar substratum instead of above its surface.

PYCNOSPORE FORMATION

The pycnospores of *Septoria lycopersici* are filiform, curved or straight, and their walls are thin. They are pluriseptate, consisting of 3 to 9 cells, and their ends are pointed to globose. Protoplasmic contents are vacuolated, but the cytoplasm itself appears smooth and homogeneous. The pycnospores range in size from 60–120 × 2–4 μ.

Progressive stages in the formation of pycnospores are shown in figure 1, C–F. Cells forming the undifferentiated sporogenous tissue at the periphery of the pycnidial cavity first push forth a small, short, papillate protrusion (Fig. 1, C). This protrusion continues to elongate (Fig. 1, D and E), and, ultimately, as the pycnospore approaches maturity, separation from the sporogenous cell occurs by basal constriction (Fig. 1, F). After a pycnospore is constricted, other pycnospores may form from the same sporogenous cell. Thus, a continuous basipetal formation of pycnospores occurs, filling the entire pycnidial chamber.

PYCNOSPORE GERMINATION

Levin studied the pycnospore germination of *Septoria lycopersici* in hanging-drop cultures of sterile water and on various media. No comparative studies were made of germination on the host plant.

With this purpose in view, germination studies were made of pycnospores in sterile water in Van Tieghem cells and also in sterile water on both the upper and lower leaf surfaces. The hanging-drop cultures were placed in humidity cases with the plants, so that temperature conditions were the same in all instances. The pycnospores were examined at the end of 24, 36, and 48 hours.

A few pycnospores exhibited initial stages of germination at the end of 36 hours. Germination was quite general at 48 hours, and this time best represents that required for germination. Even then, however, pycnospores were observed that showed no signs of germination, while others were just exhibiting the formation of germ tubes.

The first noticeable characteristic to be observed during germination is a swelling of the pycnospore due to imbibition of water. Swelling is greater for diameter than for length of the pycnospore, and a germinating pycnospore usually has a diameter 2 to 4 times greater than that of a nongerminating one. A vacuolated condition of the protoplasm is also a prominent feature during early germination.

Germ-tube formation occurs on both the inner and apical cells of the pycnospore (Fig. 3). Apical cell germination, however, is more nearly

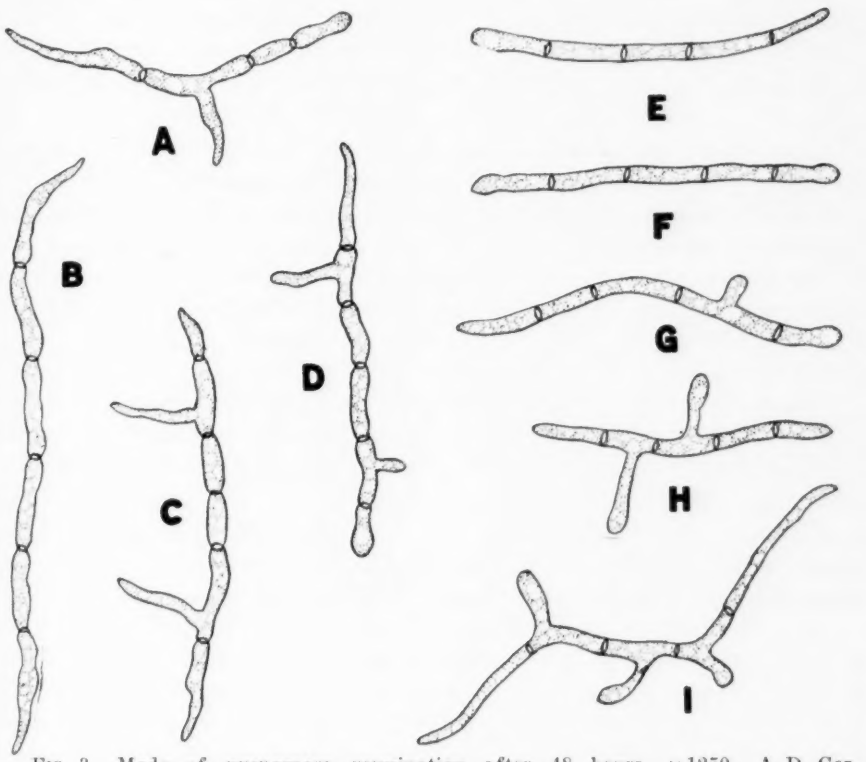


FIG. 3. Mode of pycnospore germination after 48 hours. ×1250. A–D. Germination in a Van Tieghem cell. E–I. Germination on the under side of a tomato leaf. Germ tubes of these pycnospores are rather blunt as compared with those produced by pycnospores germinated in hanging-drop culture, and the septa are not so prominently constricted.

comparable to a simple hyphal extension or elongation of the cell, since the apical cells were never observed to form lateral germ tubes. Elongation of apical cells usually is preceded by a globose enlargement at the end of the cell, and lateral germ-tube formation is characterized by a lateral wall protuberance that ultimately extends forth as a short germ tube.

Various combinations in the formation of lateral germ tubes may occur during germination. Either one cell or several cells of the pycnospore may form a germ tube, and these germ tubes may occur on the same side or on both sides of the pycnospore.

Anastomosis between 2 germinating pycnospores was frequently observed, and the anastomosing tube formed between any of the inner cells of the 2 pycnospores.

The mode of pycnospore germination, as observed during this study, is essentially the same as that reported by Levin (6). Some differences were to be noted, however, between the germination of pycnospores in

hanging-drop culture and those germinated on the host plant. As may be seen in figure 3, A to D, which represents pycnospore germination in hanging-drop culture, the germ tubes usually are characterized by rather pointed or tapering ends. The individual cells of these pycnospores are conspicuous and impart a decidedly jointed appearance to the pycnospores. The germ tubes of pycnospores germinated on the host plant (Fig. 3, E to I) are quite rounded and rather blunt. Constriction at the septa is lacking in these pycnospores.

No difference was to be noted between the germination of pycnospores on the upper and lower leaf surfaces.

INFECTION PENETRATION

The mode of infection penetration of *Septoria lycopersici* has been determined as stomatal (Fig. 1, G), as Levin (6) likewise reported. In no instance was direct infection penetration observed to occur through the cuticle and epidermal cells.

It has not been possible to follow the progressive development of the mycelium through the leaf, but the chief facts of the relationship between the fungus and the host tissue have been determined.

The mycelium is intercellular (Fig. 1, G and H), and penetrates the host cells by means of blunt, globular haustoria (Fig. 1, H). Haustoria have been observed only on the hyaline type of mycelium, and their protoplasmic contents are densely granular. The penetration hypha, after entering the substomatal chamber (Fig. 1, G), may branch and effect immediate haustorial penetration of adjacent cells. Again, the mycelium may begin intercellular extension through the leaf after infection penetration. The mycelium occurs in all parts of the leaf, but it is most abundant in the mesophyll region. Also, it is not wholly confined to the visible leaf-spot area, but extends short distances into the adjacent tissue.

Vascular tissue restricts mycelial progress to a certain extent, and hyphae sometimes terminate in this region, as shown by the hypha (Fig. 2, C) just beneath the pycnidial primordium. Hyphae, however, may occur also in the tracheae. One vessel was observed that was almost completely filled by the coiled hyphal strand. In view of the fact that direct cell penetration by the fungus does not occur, the mycelium evidently gained access to the tracheal vessel through the pits, though this point has not been verified.

The continued progress of the fungus also is frequently impeded by the large clusters of druses present in the tomato leaf.

SUMMARY

The mycelium of *Septoria lycopersici* consists of a hyaline, thin-wall type, and a brown, thick-wall type.

The mode of pycnidial formation is symphyogenous. The pycnidial cavity first forms by a schizogenous process, but, prior to pycnospore formation, a lysigenous action also is involved.

Ostiole formation occurs by tensional strain of the pycnidial wall on the leaf epidermis, and by a pressure of the pycnospores on the pycnidial wall. A lysigenous action also may occur but it was not observed.

Pycnospore formation occurs by basipetal elongation of the sporogenous cells without intervening conidiophores. The pycnospore is separated from the sporogenous cell by a basal constriction.

Pycnospore germination occurs by the formation of a lateral germ tube from the inner cells, and by elongation of the apical cells.

Infection penetration is stomatal. The mycelium is intercellular, and cell penetration is effected by means of haustoria.

DEPARTMENT OF BOTANY,
UNIVERSITY OF ILLINOIS,
URBANA, ILLINOIS.

LITERATURE CITED

1. ARCHER, W. A. Morphological characters of some Sphaeropsidales in culture. Ann. Mycol. 24: 1–84. 1926.
2. DAVIS, W. H. Single spore isolation. Proc. Iowa Acad. Sci. 37 (1930): 151–159. 1931.
3. DICKSON, B. T. The differential staining of plant pathogen and host. Science, n.s., 52: 63–64. 1920.
4. DIEDICKE, H. Die Gattung Septoria. Ann. Mycol. 10: 478–487. 1912.
5. KLEBAHN, H. Untersuchungen über einige Fungi Imperfecti und die zugehörigen Ascomycetenformen. Ztschr. Pflanzenkrank. 18: 5–17. 1908.
6. LEVIN, E. The leaf-spot disease of tomato. Mich. Agr. Exp. Sta. Tech. Bul. 25. 1916.
7. POTEBNIA, A. Beiträge zur Micromycetenflora Mittel-Russlands. Ann. Mycol 8: 42–93. 1910.
8. VAUGHAN, R. E. A method for the differential staining of fungous and host cells. Ann. Missouri Bot. Gard. 1: 241–242. 1914.
9. WEBER, G. F. Septoria diseases of wheat. Phytopath. 12: 537–585. 1922.

A PHYTOPHTHORA DISEASE OF PEACH SEEDLINGS[1]

JOHN C. DUNEGAN

(Accepted for publication December 21, 1934)

On June 6, 1929, a nurseryman at Bentonville, Arkansas, called the writer's attention to a stem canker and seedling wilt of peach trees in the nursery rows. Nonseptate mycelium was observed in the diseased tissues and an organism, subsequently identified as *Phytophthora cactorum* (L. and C.) Schröter, was isolated readily in pure culture from the diseased seedlings.

Smith (6), in 1915, and subsequently, Smith and Smith (7), described a dying back and gumming of peach and other deciduous nursery stock in California caused by *Pythiacystis citrophthora* and fungi of the *Phytophthora cactorum* group. In 1931 Arnaud and Arnaud (1, p. 1225) observed a wilting of peach trees growing in a greenhouse at Versailles, France. They found a species of Phytophthora present in most of the specimens they examined.

The disease under discussion differs in several respects from these previously described ones. In Arkansas the peach seedlings are attacked and destroyed soon after they appear above ground in the spring. In California the disease apparently has not been observed on seedlings in the spring but a similar malady has been observed on budded nursery stock. The nursery stock is infected in the late fall or winter either before the trees are dug or while they are heeled in. In France the disease developed on grafted trees.

SYMPTOMS

The first symptom of the disease is the appearance of a small, light brown lesion on the stem of the young tree, well above the soil line. The lesion enlarges rather rapidly, generally encircling the stem and develops into a brown, water-soaked canker from 2 to 10 cm. long (Fig. 1, A), which may extend downward to the soil line. The tissues of the affected region are sunken and frequently there is a profuse flow of gum through fissures (Fig. 1, B) in the epidermis.

In the early stages of the disease the leaves appear normal, but as the canker encircles the stem the terminal leaves develop a red discoloration and fail to unfold properly. These symptoms appear only on the terminal leaves.

The root system remains healthy during the early stages of the disease and the root discoloration that develops after the top dies is a secondary effect. The disease is distinctly a disease of the aerial parts.

[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Arkansas Agricultural Experiment Station.

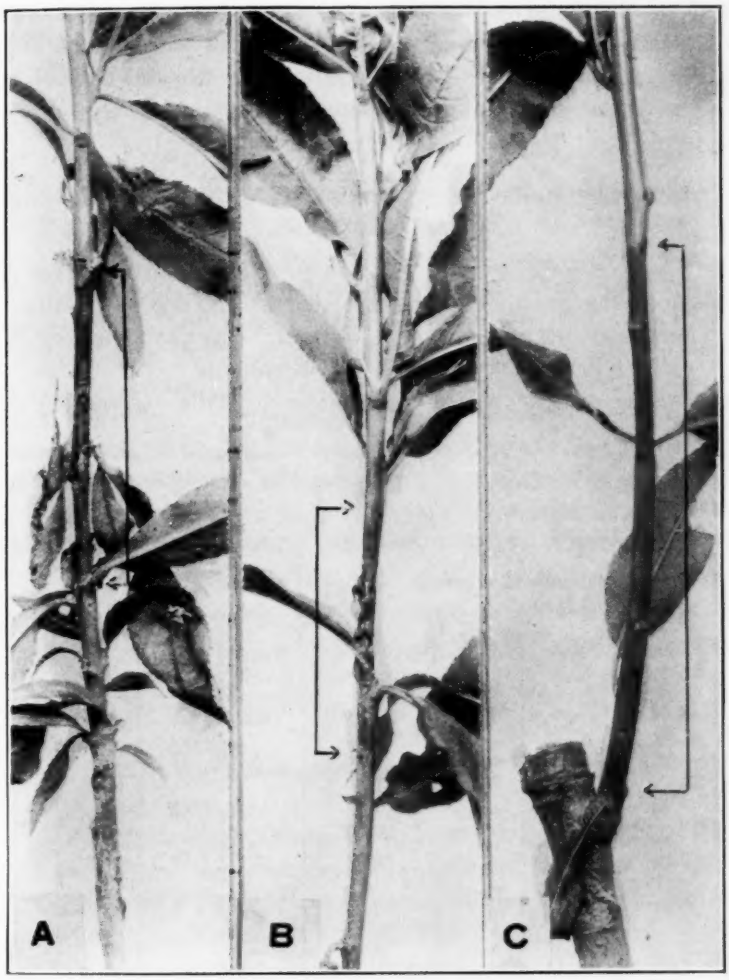


FIG. 1. A. Phytophthora canker on peach seedling. B. Another specimen showing gum oozing from necrotic region. C. Phytophthora canker on a budded tree. The bud was inserted in the fall of 1928 and the shoot was infected after growth started in 1929. All three specimens collected at Bentonville, Arkansas, June 8, 1929. Limits of cankers indicated by brackets.

A few cases have been observed in which the shoot developing from a bud that had been inserted in the fall was infected the following spring (Fig. 1, C). The symptoms on the shoots of the fall-budded trees are the same as those on the seedlings, except that there is a tendency for the shoots to fall over when the final stage of the disease is reached. The infected seedlings do not fall over, as they are supported by adjacent healthy seedlings until the lesions dry out and the tissues become stiff.

Although the majority of the infections seen by the writer developed on the main stem of the seedlings, several cases were observed in 1930 where the initial symptoms appeared on the young branches and none on the main stem. The causal organism was isolated from these cankers as readily as from those on main stems.

THE INFLUENCE OF WEATHER CONDITIONS ON THE OCCURRENCE OF THE DISEASE

It is a well established fact that the prevalence and destructiveness of phytophthora diseases are closely correlated with excessive amounts of rainfall and numerous periods of cloudy weather. The phytophthora disease of peach seedlings is no exception to this general rule.

The weather records (9) for the Bentonville, Arkansas, station (Table 1)

TABLE 1.—*Total precipitation and number of days with .01 inch or more precipitation during the months of April, May and June, 1927 to 1934, inclusive, at Bentonville, Arkansas*

Year	Precipitation in inches				Number of days with .01 inch or more precipitation			
	April	May	June	Total for the three months	April	May	June	Total for the three months
1927	9.50	4.38	7.47	21.35	16	10	12	38
1928	9.85	6.62	13.81	30.28	9	11	19	39
1929	7.85	9.93	8.05	25.83	15	16	14	45
1930	2.46	5.82	4.74	13.02	13	13	6	32
1931	4.16	5.42	3.01	12.59	10	9	7	26
1932	2.09	0.90	5.28	8.27	7	4	16	27
1933	6.93	9.62	0.14	16.69	10	14	1	25
1934	2.07	2.97	2.53	7.57	7	9	6	22

show excessive amounts of precipitation and numerous rainy days for the months of April, May and June, 1927 to 1929 inclusive and a decrease in the subsequent years. The outbreak of the disease under discussion in this paper began in 1927 and continued until 1930, although it was much reduced in severity in 1930. Since that time the disease has not been observed in northwest Arkansas and it is evident that its prevalence is closely correlated with periods of excessive precipitation and rainy weather. The data for

1928 are particularly interesting, for it was then that approximately 50,000 young plants were destroyed in one nursery.

ISOLATION AND DEMONSTRATION OF THE PATHOGENICITY OF THE CAUSAL AGENT

Pure cultures of a species of Phytophthora were readily secured from peach seedlings by aseptic removal of fragments of the inner tissues of young cankers and placing them in Petri dishes of solid corn-meal agar.

The first series of experiments to demonstrate the pathogenicity of the fungus isolated from cankers on peach seedlings was carried out at various intervals from July 18 to September 16, 1929. In these experiments small pieces of agar bearing mycelium were inserted in slits made with a flamed scalpel in the epidermis of succulent branches of the seedlings. No inoculum was inserted in the slits used as checks and all the branches were covered with frequently moistened sterile cotton. Previous to making the slits all the branches used were thoroughly swabbed with bichloride of mercury and then washed with sterile water.

The inoculum was inserted in 47 branches and cankers (Fig. 2, A) and developed on 28 branches. The first symptoms appeared 3 to 5 days after the inoculations were made; necrotic lesions 2.0 to 5.0 cm. long eventually developed on the shoots, but the parts above the lesions did not wilt. The fungus was reisolated from a number of these lesions. The noninoculated, control slits, 22 in number, healed normally.

In the second series of inoculation experiments the inoculum was inserted in slits made approximately 1 inch above the soil line on the main stem of 4 peach seedlings on July 18 and 2 seedlings on July 22, 1929. Cankers (Fig. 2, B) developed on the stems of 5 of these seedling trees above the slits, but the trees did not wilt. The noninoculated slits on 5 additional seedling trees, used as controls, healed normally.

The inoculation experiments performed in 1929 demonstrated that the fungus was pathogenic when introduced into the tissues through wounds. Cankers formed about the site of the inoculation in many cases, but the wilting so typical of the disease in the field did not develop following the inoculations.

In 1930 it was found that the fungus would grow on moistened bran; and on March 17, 1931, bran, previously inoculated with a culture reisolated from a canker produced in the 1929 experiments, was mixed with sand and placed over the soil of a pot containing 2 seedling peach trees growing in a greenhouse. One of the seedlings was a year old but was still rather succulent, while the other seedling was just a few weeks old. A mixture of sterile bran and sand was placed on the soil of another pot containing 2

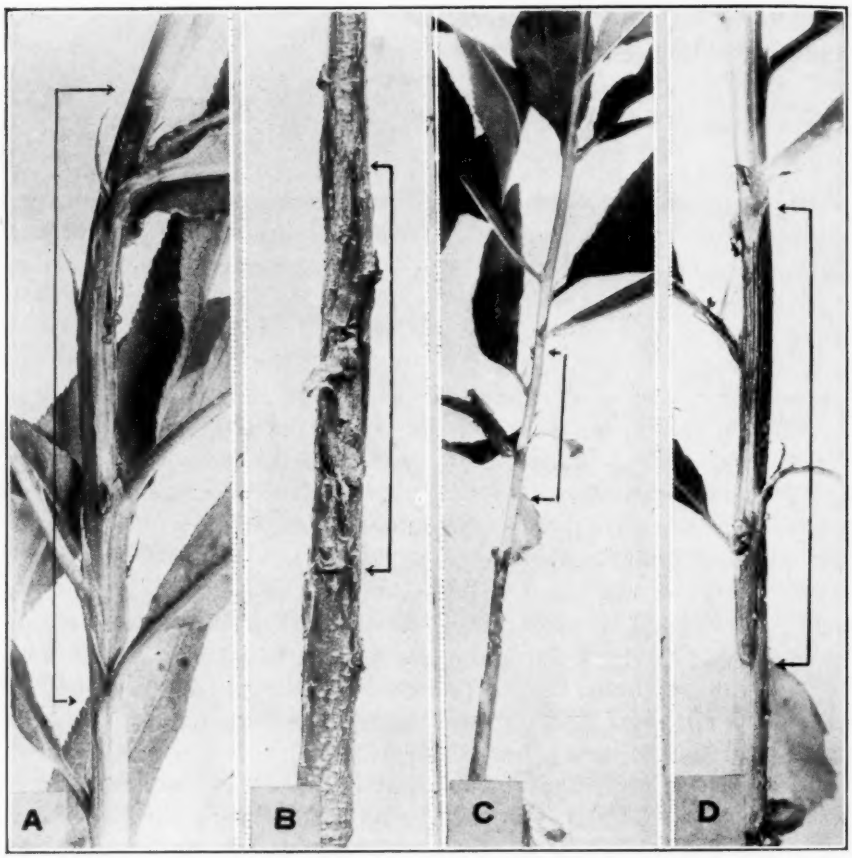


FIG. 2. A. Canker produced by inserting mycelium in a slit made in the epidermis of a succulent shoot of a peach seedling. Inoculated September 16 and photographed September 24, 1929. B. Canker produced by inserting mycelium in a non-succulent stem of peach seedling. Inoculated July 18 and photographed August 26, 1929. C. Canker produced by placing mixture of inoculated bran and sand around young seedlings on April 16, 1931. Photographed April 20, 1931. D. Portion of C enlarged to show invaded region. Limits of cankers indicated by brackets.

small seedling trees to serve as checks. The 2 pots were placed in an inoculation chamber for 24 hours and then returned to a greenhouse bench.

Water-soaked streaks were observed March 19 on the stem of the younger seedling in the pot covered with inoculated bran, and the stem and leaves were slightly flaccid. The seedlings of the same age in the control pot were turgid. At 8 A. M. the following day the infected seedling was turgid again, but 2 leaves at the apex had collapsed, and by 10:30 A. M. the seedling had wilted. The partial recovery during the night is of interest because, in

1929, during periods of rainy weather, many seedlings in the nursery rows were observed to be girdled but the tops remained turgid until the advent of clear weather, when they wilted rapidly.

The succulent year-old seedling showed no pathogenic symptoms until March 21, when a water-soaked spot appeared on the stem 1.0 cm. above the layer of bran and soil. Three days later the lesion was 9.0 cm. long and had girdled the stem. The tree wilted subsequently and was dead on March 31. Pure cultures of the organism were isolated from this plant. The 2 control seedlings remained healthy.

On April 16 a flat containing 22 peach seedlings was inoculated by covering the soil with a mixture of inoculated bran and sand. The fungus used in this experiment had been isolated from the year-old seedling in the experiment of March 17. The soil of a pot containing 10 peach seedlings was covered with a mixture of sterile bran and sand to serve as a control. The flat and the pot were placed in a saturated atmosphere for 48 hours and then returned to a greenhouse bench.

Typical cankers (Fig. 2, C and D) developed on the stems of 5 of the seedlings within 4 days and subsequently 3 more seedlings became infected. The leaves of the infected seedlings developed the reddish color and peculiar folded condition noted in the field, but wilting did not develop. The control seedlings remained healthy.

The 1931 experiments demonstrated that the fungus, reisolated in 1929 from inoculated plants, could infect noninjured seedlings with the production of symptoms identical to those observed in the nursery rows, although wilting and subsequent death of the seedlings was reproduced experimentally in only 2 instances.

DESCRIPTION OF THE CAUSAL ORGANISM

The mycelium of the fungus consists of long, profusely branched filaments devoid of cross walls except when old, and ranging in diameter from 2 to 10 microns. In young cultures the hyphae are full of yellowish, granular protoplasm, which flows rapidly through the filaments. As the cultures age the older filaments lose most of their contents and appear as hyaline, empty tubes.

The sporangia are produced sympodially on slender branches (sporangiophores) of the stout hyphae. They show considerable variation in size and shape but generally are ovate in form, measuring 30 to 45 μ long and 24 to 36 μ in diameter. Each sporangium has a small papilla and the internal contents are densely granular. On repeated occasions they have been observed to germinate to form a germ tube that, in turn, may give rise to secondary sporangia, but efforts to induce zoospore production have not been successful.

Antheridia, oogonia, and oospores develop profusely on corn-meal agar and Lima-bean agar, frequently appearing within 48 hours when vigorously growing cultures are used. The oogonia are subspherical, 32 to 38 μ in diameter, and hyaline to pale yellow. The antheridia, which vary in shape from curved tubes to spherical bodies, are predominately paragynous (of a total of 204 antheridia examined only 2 appeared to be amphigynous).

The oospores are thick-walled, 25–30 μ in diameter, yellowish, and are borne within the oogonia.

The organism grows readily on various media, such as corn-meal, Lima-bean, malt-extract and dextrose agars and has also been cultured in flasks of steamed bran for use in inoculation experiments. The amount of aerial mycelium produced varied with the kind of medium and was more abundant on Lima-bean and corn-meal agars than on dextrose and malt-extract agars. While the amount of growth and number of both sporangia and oogonia vary with kind of medium used, the length of time during which any particular transfer had been maintained in artificial culture also is an important factor. Cultures that ceased to produce oogonia could be induced to form them again by inoculating apples with the culture and reisolating from the apple tissue. The fresh isolation would frequently produce oogonia within 48 hours. The organism grew slowly at 5° C., vigorously at 21° to 26°, but was inhibited at 32°. One isolation held at 32° for 10 days and then returned to 21° made little growth at the lower temperature, while another isolation was killed by exposure at 32°.

The hydrogen-ion requirements of the organism were tested on a solid synthetic medium[2] to which soluble starch had been added as a source of carbohydrates. On this medium at 24° C. the organism grew in a range from pH 4.0 to pH 9.0.

TAXONOMY

As the result of the comprehensive studies of the genus Phytophthora initiated by Rosenbaum (5) in 1917 and subsequently carried on by Leonian (3), Leonian and Geer (4), and more recently by Tucker (8), it is possible to identify a new isolation of Phytophthora with some degree of certainty.

Probably the most outstanding characteristic of the fungus isolated from peach seedlings is the prompt and profuse production, in culture, of oogonia with paragynous antheridia. This feature limits the species to be considered to *Phytophthora cactorum* and *P. syringae*, for these are the only species that have predominately paragynous antheridia and that produce oogonia promptly and abundantly in culture. Some investigators might include *P. citricola* and *P. hibernalis*, but the first mentioned form is now

[2] Monobasic ammonium phosphate 1.0 g., potassium chloride 0.2 g., magnesium sulphate 0.2 g., soluble starch 20 g., agar 25 g., distilled water 1000 ml.

considered to be synonymous with *P. cactorum,* and *P. hibernalis* with *P. syringae.*

Phytophthora cactorum and *P. syringae* are closely related forms but, as Tucker (8) and Chester (2) have pointed out, *P. syringae* does not grow in culture at 25° C., while *P. cactorum* thrives luxuriantly at this temperature and is not inhibited until a temperature of 32° is reached. There are corresponding differences in regard to the hydrogen-ion concentration, the growth range for *P. syringae* being pH 3.5 to 7.5, and for *P. cactorum* pH 3.0 to 10.0. According to Tucker (8) the papillae of *P. syringae* sporangia are flattened, while those of *P. cactorum* protrude and often are inconspicuous. Sporangia from corn-meal agar cultures of the peach-seedling organism had small protruding papillae of 1.5–2.0 μ in length.

The peach-seedling fungus in culture, as mentioned previously, is marked by the profuse production of oogonia with paragynous antheridia. The sporangia bear protruding papillae, the organism grows vigorously at 26° C. and not at 32°, and its hydrogen-ion range is from pH 4.0 to at least pH 9.0. Since these are characteristics of *Phytophthora cactorum,* it is concluded that the organism isolated from the stem cankers on peach seedlings is this species.

PATHOLOGICAL HISTOLOGY

Sections were prepared from the cankers produced in the inoculation experiments of March 17, and April 16, 1931. This material was used in preference to that from the nurseries, as the latter was found unsatisfactory for histological work because of adhering soil particles.

The sections show that the fungus affects all the tissues from the epidermis to the cambium. The cells of the epidermis and the parenchyma beneath it collapse into a dark colored mass. The mycelium of the fungus was observed among the distorted cells of the cortical parenchyma but no oospores were observed. Fragments of older cankers were dissected to ascertain whether oospores developed in the tissues as the disease progressed, but they were not found even in this older material.

The cells of the cortical fibers are discolored but retain their shape. Their presence, however, offers no barrier to the progress of the fungus, as the cells of the phloem and cambium were completely destroyed in most of the sections. The space formerly occupied by these tissues is filled with a series of gum pockets and only fragments of the former cellular structure are visible. The mycelium of the fungus has been observed along the boundaries of the gum pockets and in a few instances fragments of the mycelium were detected in the gum pockets. The youngest cells of the xylem adjacent to the cambium are discolored but, on the whole, the xylem and pith appear uninjured by the fungus.

CONTROL OF THE DISEASE

The only known practical control measure is to avoid growing seedlings in poorly drained areas. This procedure does not eliminate the possibility of the disease appearing in the nursery if environmental conditions are exceptionally favorable, but a number of nurserymen have told the writer that they have reduced the loss to a negligible figure by selecting well-drained sites for their seedling nurseries.

The low value of an individual seedling in a planting of 50,000 to 100,000 seedlings, the extreme susceptibility of the peach to spray injury, and the variation in prevalence of the disease in various parts of a nursery are factors that make the problem of disease control in the nursery a very difficult one.

In 1930 the writer attempted to control the phytophthora disease of peach seedlings by applying such materials as aluminum sulphate, zinc sulphate, flowers of sulphur, ground flour of sulphur, and a monosulphide of sulphur to the ground along the nursery rows close to the trees. These materials are relatively inexpensive, and by applying them to the ground instead of spraying them on the trees the risk of injury to the foliage was eliminated. When the results were recorded on June 6, 1930, it was found that, while the non-treated control plot showed 5 per cent of the seedlings affected, the disease was present in the treated plots in varying degrees, ranging from 1 to 26 per cent. This irregular distribution of the disease in plots selected at random completely obscured the recognition of any beneficial results that might have been secured by the various treatments.

The absence of the disease since 1930 has prevented further experiments on this phase of the problem.

SUMMARY

A disease of peach seedlings caused by *Phytophthora cactorum* and characterized by the formation of a canker on the succulent stems, a wilting of the parts above ground and the ultimate death of the young trees, has been observed in northwest Arkansas.

The fungus attacks the seedlings soon after they emerge above ground in the spring.

The symptoms of the disease are described in some detail. The disease is confined to the aerial parts, the root system remaining healthy until the death of the aerial parts ensues.

The occurrence of the disease is favored by abundant rainfall and cloudy weather during the early part of the growing season.

Pure cultures of an organism identified as *Phytophthora cactorum* were readily secured from the diseased tissues. The characters of the fungus in culture are briefly discussed. The pathogenicity of the fungus was proved

by a series of inoculation experiments culminating in the production of typical wilt of the experimental plants growing through a mixture of sand and inoculated bran.

A study of the cankers shows that the effects of the fungus are confined to the cortex and cambium. Gum pockets are formed in the region of the phloem, while the outer parenchyma tissues collapse into a dark discolored mass resting on the cortical fibers.

The only practical control measure known is to grow the seedlings in well-drained sites.

COLLEGE OF AGRICULTURE,
UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARKANSAS.

LITERATURE CITED

(1) ARNAUD, G., and M. ARNAUD. Traité de pathologie végétale. Tome I. Vols. I, II, et Atlas. 1900 pp. Paul Lechevalier et fils, Paris. 1931.

(2) CHESTER, K. S. A comparative study of three Phytophthora diseases of lilac and their pathogens. Jour. Arnold Arb. **8**: 232–268. 1932. [Original not seen.]

(3) LEONIAN, L. H. Physiological studies on the genus Phytophthora. Amer. Jour. Bot. **12**: 444–498. 1925.

(4) ————, and H. L. GEER. Comparative value of the size of Phytophthora sporangia obtained under standard conditions. Jour. Agr. Res. **39**: 293–311. 1929.

(5) ROSENBAUM, J. Studies of the genus Phytophthora. Jour. Agr. Res. **8**: 233–276. 1917.

(6) SMITH, E. H. Pythiacystis infection of deciduous nursery stock. Phytopath. **5**: 317–322. 1915.

(7) SMITH, R. E., and E. H. SMITH. Further studies on pythiaceous infection of deciduous fruit trees in California. Phytopath. **15**: 389–404. 1925.

(8) TUCKER, C. M. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp. Sta. Res. Bul. 153. 1931.

(9) U. S. Department of Agriculture, Weather Bureau. Climatological Data, Arkansas Section. April, May and June 1927 to 1934 incl. Little Rock, Ark.

SUMMARY OF INVESTIGATIONS WITH USTILAGO STRIAEFORMIS PARASITIZING SOME COMMON GRASSES

W. H. DAVIS

(Accepted for publication November 30, 1934)

Some of our common grasses are parasitized by a leaf smut (*Ustilago striaeformis* (Westd.) Niessl). This species is an assemblage of species due to the fact that previous investigators have classified the organism according to morphological characters. Mycologists were unable to find consistently varying morphological spore characters and symptoms of the smut in the various hosts, so they assigned the smut in each grass host to the same species. Furthermore, they were unable to consider the biology of the fungus because they did not have the spore germination under control for inoculation experiments and life-history studies. The conditions of spore germination, however, have been solved (2), the life-history described (3), and two physiologic forms reported (4), one in timothy, *Phleum pratense* L., and another in redtop, *Agrostis palustris* Huds. The biology, however, of the American forms of the smut in orchard grass, *Dactylis glomerata* L., Kentucky bluegrass, *Poa pratensis* L., and annual bluegrass, *P. annua* L., has remained unsolved.

Liro (5: 317, 377, 380) believed that the original specimen of *Ustilago striaeformis* in *Holcus lanatus* L. was none other than the uredo form of *Puccinia dispersa* Eriks. and Henn., and the host was *Triticum repens* instead of *Holcus lanatus.* He does not, however, wish to consider his examination of *exsiccati* as final, since a sufficient number of distributed specimens was not available for his examination. Nevertheless, Liro's researches cause one to doubt the advisability of assigning these smuts in some of our agricultural grasses to the one species, *U. striaeformis,* and they have shown need for further investigation.

Liro (5: 372, 379, 392) reported leaf smut on *Dactylis glomerata* in Europe as *Ustilago salveii* Berk. and Broom. His investigations regarding *U. salveii* showed that it is a distinct morphological species possessing spores smaller than those of the typical *U. striaeformis;* 8×12 as compared with 10×15 μ. Furthermore, *U. salveii* was biologically fixed to its host plant, since it neither infected species of Phleum, Festuca, Poa, nor Agrostis, growing in the same garden with infected *Dactylis glomerata,* nor did it infect inoculated species of Festuca, Anthoxanthum, Aira, Arrhenatherum, Calamagrostis, *Agrostis canna* and *A. vulgaris, Avena elatior, Alopecurus* and Milium.

It is noteworthy that Liro found the spores germinating in nature during August and no after-ripening period of 254 days was reported,

as occurs in our American species (2). Besides, he did not inoculate timothy, redtop, Kentucky blue, and annual bluegrasses, thus leaving the biologic relation of the smut in these grasses and the striped smut in our orchard grass unsolved. He also raised the question as to whether the smut in the American species of orchard grass is a physiological form of *U. striaeformis, U. salveii* or a definite species as yet unnamed.

No published investigations regarding the biology of *Ustilago striaeformis* in *Poa pratensis* and in *P. annua* are known to the writer and it is obvious that these should be investigated. With the above information at hand, the writer undertook by investigation to answer the following questions:

1. Is *U. striaeformis* a single species or an assemblage of several species of smut fungi that parasitize many of our grasses?

2. What Latin binomial should be assigned to the leaf smut of orchard grass? Should it be referred to *U. salveii, U. striaeformis,* or a new species of Ustilago?

3. Are there physiologic forms of *U. striaeformis* on *Dactylis glomerata, Poa partensis,* and *P. annua?*

METHODS AND MATERIALS

The methods employed were essentially those already described in a previous report (4). The materials also were assembled, the spores stored for ripening, and the grass seeds cultured according to the method previously employed (4). There were, however, some additions and variations that should be presented.

Dactylis glomerata, infected with the leaf smut, was difficult to locate. From 1921 to 1933, the writer has made field observations in Iowa, Wisconsin, Illinois, New York, Pennsylvania, and in each of the New England States. New York is the only State in which this smut could be located as parasitizing this grass. Furthermore, specimens of this smut have been solicited repeatedly from pathologists, but not one new station has been located by them and reported. So the 3 New York State stations located at Ithaca, Loon Lake, and Elmira, respectively, are the only natural stations known to the writer at this time. *Dactylis glomerata* plants bearing smutted leaves were removed from one of these stations and transplanted in the pathological garden at the Massachusetts State College. Furthermore, spore materials were collected from each of these 3 stations and the spores properly stored for ripening. When tests showed a 35 per cent germination or better, the spores were employed for inoculating seedlings of orchard grass. Plants infected by artificial inoculation were transplanted and retained in the pathological garden for 5 years.

They furnished a sufficient supply of spore materials each year, since the fungus was perennial within them.

Inoculated plants of *Dactylis glomerata* often were retained in the plots for 2 years before the first symptoms of the smut were observed. The symptoms generally showed best in matured plants during the early spring and the autumn.

Seed of *Dactylis glomerata* was obtained from two reliable seedsmen, but the agronomic strains were unknown. Five collections also were obtained from matured field plants under cultural conditions in Massachusetts. Only two of these, however, were used in the inoculating experiments, since no strain seemed immune from this smut fungus.

Poa pratensis infected with leaf smut was located in Wisconsin and New England. Amherst, Massachusetts and Windsor, Connecticut, were the 2 best stations for collecting spore materials. At times, 30 per cent of the plants at these stations was infected and 85 per cent of the infected seedlings often killed under adverse host conditions that afterwards developed. Spore materials were collected from infected hosts with partly matured panicles, from immature plants, and from seedlings. The spore materials were incubated and the spores ripened within periods varying from 110 to 250 days. The percentage of germination, however, was rather low, varying from 35 to 60. The seedlings that were inoculated had been cultured from seeds purchased of 2 reliable seedsmen located in Massachusetts.

Smutted plants of *Poa annua* were more difficult to locate than those of *P. pratensis* and *Dactylis glomerata*. For definite determination of the hosts, floral parts removed from infected plants were classified by A. S. Hitchcock and his associates. Spores collected in the spring germinated in late autumn, 1929. The ripening period varied from 94 to 128 days. Only 2 lots of these spores were collected on account of the tedious work of locating a sufficient quantity of spore materials for inoculating purposes. One lot germinated 95 per cent, following storage for 150 days under proper conditions. Thus an attempt was made to use spore materials from properly classified hosts and from as many stations as possible so as to include any physiologic race that might be confined to one locality. Furthermore, the grass seeds were unassorted, so as to include both susceptible and resistant strains of hosts, provided they existed.

INOCULATIONS

The inoculations were begun in 1928 and performed during different seasons of each year until 1934, when the experimental plots were destroyed by unscrupulous individuals. The results of the inoculation work with spores from *Dactylis glomerata, Agrostis alba, Phleum pratense, Poa*

pratensis and *P. annua* had, however, been observed and recorded. But those with sweet vernal, *Anthoxanthum odoratum* L., and other experiments were destroyed.

Only 2 grass hosts were inoculated in any one series so that both reciprocal inoculation and inoculations within the hosts species could be conveniently performed. Lots of *Dactylis glomerata* and *Phleum pratense* seedlings were each inoculated with germinated spores from both, a total of 27 times; *D. glomerata* and *Agrostis alba* were similarly inoculated in 9 series *D. glomerata* and *Poa pratensis,* 9 times; *D. glomerata* and *Poa annua* 4 times.

The observations showed smutted orchard grass plants in each series, except one, in which smut spores from *Dactylis glomerata* were employed. All inoculation of this grass with spores from other hosts failed to produce infections. The percentage of infected plants varied from 2 to 20 for those inoculated, and the incubation period varied from 8 weeks to 2 years before rugae of smut spores were observed in orchard grass leaves. These results showed that the smut in orchard grass is biologically differentiated from that in *Phleum pratense, Agrostis alba, Poa pratensis* and *P. annua.*

To determine the biology of *Ustilago striaeformis* in *Poa pratensis,* a plan similar to that already described for *Dactylis glomerata* was followed, but germinated spores of the *P. pratensis* strain were employed as the main inoculum. The reciprocal inoculations and inoculations within the host species follow: *P. pratensis* and *Phleum pratense,* 7 series; *Poa pratensis* and *Agrostis alba,* 8 series; *Poa pratensis* and *D. glomerata,* 9 series; *Poa pratensis* and *P. annua,* 3 series. The smut striae showed distinctly in the seedlings of *P. pratensis* only when inoculated with spores from that grass. All other inoculated plants remained smut-free. The percentage of infected plants varied from 0, in one case, to 60, with an average of 22 per cent for all other suscepts inoculated. These results also show that the *P. pratensis* strain is biologically different from those of timothy, redtop, orchard grass and annual bluegrass.

Smut spores of the *Poa annua* strain were similarly used for determining its biological relations to timothy, redtop, orchard grass and June grass strains. From the 2 lots of ripened spores, 3 series of inoculations of each host species and reciprocals were made. The percentages of infected *P. annua* plants for the series varied from 2 to 8 and all these were within the host species of *P. annua* that had been inoculated with spores from this grass and no infection showed elsewhere.

These results showed that the *Poa annua* strain of smut differs biologically from the *Phleum pratense, Agrostis alba, Dactylis glomerata* and *Poa pratensis* strains. Furthermore, that leaf smut in *P. pratense, A. alba, D. glomerata, Poa pratensis* and *P. annua* is in each instance a physiologic

form peculiar to its specific host, considering the conditions under which the work was performed.

FIELD OBSERVATIONS

From 1920 to 1934, the writer made field observations on *Ustilago striaeformis* and collected it in the following States: Wisconsin, Iowa, Illinois, New York, Ohio, Pennsylvania and the New England States. The observations regarding physiologic forms of the smut in the fields may be summarized:

Infected *Phleum pratense, Agrostis alba,* and *Poa pratensis* have been collected in contact with *Dactylis glomerata,* yet no infected plants of the latter were observed at those stations.

The scarcity of leaf smut in *Dactylis glomerata* would lead one to believe that it is either a physiologic form or a distinct species.

Many smutted plants of *Poa pratensis* have been found growing in contact with *Agrostis alba,* which remained perfectly healthy in that vicinity.

Also, the paucity of smutted plants of *Poa annua* affords some evidence in support of physiologic specialization, or a different species.

These observations present additional evidence of physiologic specialization of the smuts in *Dactylis glomerata, P. pratensis* and *P. annua.*

NOMENCLATURE

The question arises as to whether leaf smut of *Dactylis glomerata* should be referred to *Ustilago salveii, U. striaeformis* with its physiologic form, or to a new species. As previously stated, Liro considered *U. salveii* as a distinct species parasitizing *D. glomerata* cultured in Europe. His classification was based on the facts that the spores were smaller than *U. striaeformis* found in some other hosts and that it was "biologically fixed" to its host.

Experimental evidence presented in these data, shows that the smut on *Dactylis glomerata* in the United States also is biologically fixed to its host. However, the spores are slightly larger than those from other host species examined and considerably broader than those of *Ustilago salveii,* 10×12 μ as compared with 8×12 μ for *U. salveii* and 9×11 μ for the other grass smuts (Table 1). Nevertheless, a constant physiologic basis seems of more value in determining this species than such a variable morphological basis as spore measurements. The measurements were made from spores collected during a 2-year period and after the spores had been subjected to conditions varied by locality, ripening, germinability, desiccation, moisture, season, storage of host, position in the hosts, light, and heat. Since there was such a degree of variability for different lots of spores, biometric studies, already begun, were discontinued and only limits of variation together with arithmetical means were considered (Table 1).

TABLE 1.—*Measurements assigned to spores of some leaf smuts of grasses*

Authority	Hosts	Measurements—*U. striaeformis* (Microns)
Clinton	Grasses	9–14 long; spherical to ellipsoidal.
Davis	Grasses	9 × 11 standard; 8–11 × 9–15, variation.
Liro	Grasses	8–11, limitations for all measurements made.
	Dactylis glomerata	8 × 12. For *Ustilago salveii*, only.
Davis	" "	10 × 12, standard; 7.6–12 × 10.2–15.3, variation.
	Phleum pratense	8.8 × 11.3. Average for 8 collections.
	Agrostis alba	9.3 × 11.4. Average for 10 collections.
	Poa pratensis	8.4 × 10.4. Average for 3 collections during 2 seasons.
	P. annua	8.2 × 10.0. Average for 2 collections during 1 season.

These showed that the spores of *U. striaeformis* in *Dactylis glomerata* were larger than those from the other hosts examined. Furthermore, spores from this grass generally could be identified by the steel gray to smoky hue replacing the shades and tints of brown observed in spores from other grass hosts. Also, spores in some collections were somewhat irregular in outline and had a tendency to collect in a mass or "spore ball" that consisted of several hundred spores. In most respects, this fungus behaved differently from those of other grass hosts; so it seems best to consider this as a separate species, **Ustilago clintoniana,** sp. nov.

Rugae or black pustules of spores located between leaf traces in leaf blades, sheaths and stems of *Dactylis glomerata* cultured in New York State and Massachusetts. The host epidermis is opened and the dusty spores emerge leaving portions of the leaf blades in a shredded condition. Spores often assemble in globose to elongated masses or "spore balls," ellipsoidal and mostly regular in outline; color, a smoky brown; echinulations prominent, about 40 on one facies; spores 10 × 12 μ. Differentiated from other forms of *Ustilago striaeformis* in grass hosts by its larger, darker spores, more regular in outline and tend to cling in spore balls, attached or unattached to host tissue. When they germinate, their germ tubes are longer and the protoplasm collects in the tips. The habitat is not commonly associated with other grass smuts, as it occurs in isolated places. The fungus is biologically fixed to its host under the conditions examined. Type specimens were deposited in the Cryptogamic Herbarium of Massachusetts State College, and the accession number is 3319.

The forms of *Ustilago striaeformis* in *Poa pratensis* and *P. annua* compare favorably with those in *Phleum pratense* and *Agrostis alba* as regards spore color and behavior. Spores from these two hosts, however, were smaller than from *Phleum pratense* and *Agrostis alba* (Table 1) under

the conditions examined. These variations in size were not deemed sufficient to establish a separate species in each case in view of the fact that each was physiologically fixed to its host; they, therefore, will be considered as physiologic forms of *U. striaeformis*. The following classification is suggested for the United States forms:

1. *Ustilago striaeformis* forma *Phlei.*
   Host: *Phleum pratense* L.
2. *Ustilago striaeformis* forma *Agrostidis.*
   Host: *Agrostis palustris* Huds.
3. *Ustilago striaeformis* forma *Poae-pratensis.*
   Host: *Poa pratensis* L.
4. *Ustilago striaeformis* forma *Poae-annuae.*
   Host: *Poa annua* L.
5. *Ustilago clintoniana,* sp. nov.[1]
   Host: *Dactylis glomerata* L.

This classification is not strictly in keeping with Liro's, for he listed a separate species on each host and gave it a Latin binomial consisting of the genus Ustilago with a species significant of the host parasitized or to which it was biologically fixed (5:373). With this in mind, the first four species would then become: *Ustilago phlei-pratensis; U. agrostis-palustris; U. poae-pratensis; U. poae-annuae,* each a *species novum.*

In the future, other hosts may be found for these physiologic forms. Furthermore, undescribed physiologic forms may exist on other hosts, as only a small number of the large host list has been under experimentation.

SUMMARY

A summary of 12 years' observations and experimentation with *Ustilago striaeformis* parasitizing *Phleum pratense, Agrostis alba, Dactylis glomerata, Poa pratensis,* and *P. annua* is presented. These findings may be briefly stated:

The conditions for spore germination have been determined.

The life histories are known; infection is initiated in the seedling.

The genus has been determined; each smut belongs to the genus Ustilago, which was determined by the type of spore germination.

A form of the smut fungus is physiologically fixed to each host examined.

The smut on orchard grass is here considered a distinct species, *Ustilago clintoniana,* sp. nov.

A physiologic form is assigned to each smut parasitizing the other 4 hosts, since the spores bear resemblance to the accepted type form parasitizing timothy and often called "timothy smut" in literature.

[1] Named in honor of Dr. G. P. Clinton who was first to germinate the spores removed from redtop and definitely change *Tilletia debaryana* F. de Wald. (*T. striaeformis* Oud.) to *Ustilago striaeformis* by presenting the proper, accurate, scientific data.

LITERATURE CITED

1. CLINTON, G. W. Leaf smut of timothy, redtop and bluegrass. Ill. Agr. Exp. Sta. Bull. 57: 348–349. 1900.
2. ————————. Ustilaginales. North American Flora 7: 18–19. 1906.
3. DAVIS, W. H. Spore germination of *Ustilago striaeformis*. Phytopathology 14: 251–266. 1924.
4. ————————. Life history of *Ustilago striaeformis* which causes leaf smut in timothy. Journ. Agr. Res. 32: 69–76. Jan. 1, 1926.
5. ————————. Two physiological forms of *Ustilago striaeformis*. Phytopath. 20: 65–75. Jan. 1930.
6. LIRO, J. IVAN. Die Ustilagineen Finnlands. Annales Academicae Scientiarum Fennicae, Serie A, 17: 1–636. 1924. Helsinki, Finland.